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ON INTESTINAL ABSORPTION AND THE SALINE CATHARTICS.[1]

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IN his well-known paper on the absorption of salt solutions from the intestine Heidenhain[2] came to the conclusion that two distinct factors were involved in the process, — the osmotic pressure of the solution, and the "physiological activity" of the epithelium. The latter induces a constant current from the lumen of the bowel towards the blood vessels.

Hamburger[3] accepts Heidenhain's account of the osmotic action, but attempts to show that his "physiological activity" is really a combination of the effects of certain physical forces. These are molecular imbibition; the intra-intestinal pressure, which induces filtration; and the suction induced by the blood current through the intestinal vessels, similar to that observed with the ordinary suction pump of the laboratory. Hamburger's explanation of the intestinal absorption therefore involves an obscure process — the molecular imbibition — but differs from Heidenhain's in not involving the living cell.

Heidenhain performed a few experiments with solutions of the saline cathartic magnesium sulphate, from which he found that the water was much more slowly absorbed than from corresponding solutions of sodium chloride. He explains this retarded absorption by supposing that the sulphate lessens the "physiological activity" and that movement of its solutions is therefore controlled more by

[1] Received by the editors May 5, 1898.

[2] HEIDENHAIN: Arch. f. d. ges. Physiol., 1894, lvi, p. 579.

[3] HAMBURGER: Archiv für Physiologie, 1896, p. 428.

their osmotic pressure than is the case when solutions of non-purgative substances such as common salt are employed. In some experiments in which sodium sulphate or sodium fluoride was added to solutions of sodium chloride the absorption was also retarded for the same reason, each of these salts weakening the "physiological activity," although the fluoride is much more powerful than the sulphate.

Of the factors involved in Hamburger's scheme, only one — the molecular imbibition — can be affected by a change in the salt contained in the solution, for the filtration and the suction of the blood current remain unchanged. Hamburger made a few experiments to satisfy himself that molecular imbibition could occur in dead tissues, but a much more extensive investigation of the subject had been made earlier by Hofmeister.[1] From Hofmeister's results it would appear that colloid substances (gelatine), and pieces of dried tissue, such as the wall of the bladder, are by no means indifferent to the solutions in which they are soaked. Thus much more of a solution of sodium chloride, and of the salt itself, was imbibed than of solutions of some other salts including some of the saline cathartics.

The more recent work of Hedin[2] to which we shall return later, indicates that the red blood cells also imbibe more freely the solutions of certain salts than those of others for which they seem to have less affinity.

We felt that some light might be thrown on the process of intestinal absorption by a more accurate definition of the group of saline cathartics, and we have for this purpose compared the rate of absorption from the intestine of a large number of salt solutions with that of a one per cent sodium chloride solution. On looking over the list of salts which are generally regarded as saline cathartics it is apparent that the anion, or acid constituent of the salt, is generally the determining factor. Thus sodium sulphate, potassium sulphate, sodium phosphate, potassium tartrate, potassium-sodium tartrate, potassium citrate, sodium citrate, potassium ferrocyanide, and sodium ferrocyanide, are all looked upon as cathartics, while sodium chloride, potassium chloride, sodium acetate, potassium acetate, etc., are believed to be indifferent so far as action on the bowel is concerned. It is evident therefore that the anion or acid constituent is the deter-

[1] Hofmeister: Archiv für exper. Pathol. und Pharmakol., 1890, xxvii, p. 395; 1891, xxviii, p. 210.

[2] Hedin: Arch. f. d. ges. Physiol., 1898, lxx, p. 525.

mining factor here, for the cations K and Na occur in both groups. As regards the magnesium salts the basic constituent or cation would also seem to be involved, for while in magnesium sulphate and magnesium citrate the purgative anion might explain the effects, these salts are generally believed to be more active than the corresponding salts of the alkalies, and in addition magnesium chloride, magnesium oxide, and magnesium carbonate have also some cathartic action; the presumption is therefore strong that the Mg ion is not indifferent as the K and Na ions are.

We took up first the question of the purgative anions by comparing the rate of absorption of a large number of salts of soda. A preliminary note of our results was published in the Journal of the Boston society of medical sciences, January 18, 1898. Almost simultaneously, Höber[1] published an account of his investigations on the absorption of a number of salts, dwelling particularly upon the effect of the basic constituent. We have therefore confined our examination of the cations to a few experiments which were carried out mainly to confirm his results, which we have much pleasure in doing.

In order to compare the influence of the salts on the unknown factor in absorption, whether this be physiological activity or molecular imbibition, it is of course necessary to eliminate the influence of the known factor, osmosis, by using solutions of the same osmotic pressure. This may be most easily accomplished by forming solutions which cause an equal depression of the freezing point. Our point of departure was a solution of about one per cent sodium chloride, which gave a depression of the freezing point ($\triangle$) of 0.59 — 0.64° C., estimated by Beckmann's apparatus. The other salts employed were dissolved in water, the freezing point of each solution determined, and more water or salt added until $\triangle$ approached that of the sodium chloride solution. We are therefore unable to state the percentage composition of most of our solutions, but append the depression of the freezing point to each one (see protocols, page 429). The variation in the $\triangle$ of the solutions may at first sight seem to be considerable, but the osmotic pressure never varied more than five per cent above or below the average; and when the solution of a salt showed any marked departure from the rate of absorption of the standard sodium chloride solution, care was taken to reduce the error arising from this variation in the osmotic pressure to a minimum. As a matter of fact a considerable variation in the osmotic pressure may

[1] Höber: Arch. f. d. ges. Physiol., 1898, lxx, p. 624, issued March 3.

be allowed without causing any appreciable difference in the rate of absorption, owing to the unavoidable errors of the method.[1] Thus a solution of NaCl Δ 0.67 was found to disappear as rapidly as one of Δ 0.61.

In our first experiments we used rabbits, but we could obtain no satisfactory results, as the absorption from the intestine seems to be extremely irregular in these animals. This may be partly due to the fact that it is impossible to empty the stomach and bowel by fasting of reasonable duration, and partly perhaps to the rabbit's intestine being more sensitive to handling than that of the other animals used. The cat's intestine gave somewhat better results, but here also the absorption was often irregular. The great majority of our experiments were performed on dogs, and we have left those on the rabbit entirely out of account, while the results obtained from the cat's intestine have always been controlled by others on the dog.

The dogs and cats were anæsthetized by the subcutaneous injection of morphine, followed by the administration of chloroform acetone by the mouth. In some cases ether or chloroform was given by inhalation, instead of chloroform acetone. In every case the animal fasted for 36–48 hours before the operation, and in our later experiments we obtained the best results from animals which had fasted for three or four days. This preliminary fasting appears to be of great importance in any experiment in which a regular absorption is necessary.

In all our experiments two or more intestinal loops were used. They were ligatured off in the usual way. We are quite aware of the objections to this method, for Hay[2] showed that ligation is liable to cause irritation of the bowel. Owing to the limited supply of dogs, however, we could choose only between using several short loops, or using one long loop a number of times, and we soon found that the necessary manipulations rendered a loop very unreliable after three or four injections. We have attempted to avoid the disadvantages of our method by using a series of controls. Thus, the experiment was commenced by ascertaining the rate of absorption of the standard solution in all the loops, and this control was repeated whenever any solution was found to deviate considerably from the

[1] In Experiments 21 and 23, solutions of salts which differed considerably in their osmotic pressure were used for a purpose apart from the general scope of the work.

[2] Hay: Saline cathartics, Edinburgh, 1884.

standard solution in rate of absorption. In this way any abnormality developed in an individual loop could be recognized. In addition one loop was injected each time with the standard solution, and we could thus eliminate any errors due to the general condition of the animal, and also those arising from the manipulation, for the control loop was treated in exactly the same manner as the others. In estimating the rate of absorption of any solution we have taken into consideration not only the variation in the particular loop in which it was contained, but also any change in the control loop. Our loops were much shorter than those of most other investigators, varying from 25 to 45 cm., but it seems to us that the unabsorbed fluid can be much more completely removed from these short loops than from the longer ones, and that one source of error may thus be lessened. On the other hand, the short loops have the disadvantage that greater irritation is produced by the close proximity of the ligatures; but we believe that our system of controls has reduced very greatly the error arising from this source as well as the error due to the different rate of absorption in different parts of the bowel (Heidenhain). A glass cannula was passed into each loop, fixed by a ligature, and closed by a short indiarubber tube and clamp. In some of the early experiments the loops were washed out before and after each injection of salt solution, but we found that the additional manipulation caused a larger error than that arising from the use of unwashed loops, and this procedure was abandoned in our later work. The loops were emptied by gently stripping them, and were not exposed to the air longer than was absolutely necessary in order to empty them completely.

The salts examined fall naturally into groups as follows:

1. *The halogen salts:* NaCl, NaBr, NaI, NaFl. Exp. 17, 18, 19. Of these salts the bromide seemed to be absorbed as rapidly as the chloride, while the iodide was sometimes absorbed more slowly. In Experiment 17, however, all three salts disappeared equally from the loops, and in this experiment it is noted that a fresh iodide solution prepared that morning was used. It seems possible that the divergence in Experiments 18 and 19 was due to the use of an older solution, in which some decomposition had occurred. Höber found the chloride the most easily absorbed of the three, then the bromide, and last the iodide. In our experiments we did not observe such marked differences in the rate of absorption as appear in his protocols, and in fact are inclined to hold that very little difference exists between these salts in regard to the rate of absorption of their solutions. The

fluoride, on the other hand, is absorbed with great difficulty, as is shown in Experiment 19 and in others not included in the published protocols. It always caused more or less congestion and inflammation of the loop, as is evidenced by the absence of absorption of the standard solution from the loop afterwards. Heidenhain also found that the presence of even a small percentage of fluoride in a salt solution retarded its absorption. The chloride, bromide, and iodide may thus be classed among the indifferent salts, while the fluoride has a very distinctly retarding effect on absorption.

2. *Other inorganic salts :* Na_2SO_4, $Na(C_2H_5)SO_4$, $NaNO_3$, Na_2HPO_4, NaH_2PO_4, $MgSO_4$, KNO_3, $K(C_2H_5)SO_4$,[1] $(NH_4)_2SO_4$. Experiments 1, 2, 9, 10, 12, 17, 18, 20, 21, 22, 23, 24. The simple sulphates were absorbed much more slowly than the chlorides, as has been observed by a number of workers on the subject. No evidence of inflammatory reaction was observed, and the loop rapidly returned to its normal condition when the solution was removed. Sodium ethyl-sulphate has been used occasionally as a mild saline purge, and from our experiments it would seem to stand midway between sodium chloride and sodium sulphate. No simple sulphate was contained in the fluid injected or in the residue. The nitrate solutions are more slowly absorbed than the chlorides, but much more rapidly than the sulphates, as was observed also by Höber. The use of the nitrates was followed in one of our experiments (Exp. 18) by very distinct signs of irritation and inflammatory reaction, the residue being blood-stained and containing large quantities of mucus. The nitrates are generally looked upon as being more irritant to the alimentary canal than such salts as the chlorides and sulphates, and although in the second experiment (Exp. 24) no signs of irritation of the bowel were present, we think it questionable whether the fluid found at the end of the experiment was really due to the lack of absorption or to an effusion into the bowel. The two phosphates proved almost identical in the rate of absorption, and may best be classed with sodium sulphate.

3. *Ferrocyanides and ferricyanides.* Experiments 21, 22, 23. These two salts seem to be absorbed as slowly as the sulphates. The ferricyanide solution contained no ferrocyanide when injected, but the residue gave a copious precipitate of Prussian blue, indicating that much of the ferricyanide had been reduced to the ferrocyanide. The effect is therefore probably due to the latter salt.

[1] This salt was found to contain a considerable quantity of ordinary sulphate.

4. *Salts of the fatty acids:* sodium formate, acetate, propionate, butyrate, valerate, caproate, œnanthylate, and caprylate. Experiments 4, 7, 11, 13, 14, 15, 16, 19. The first six of these salts are absorbed as rapidly as the chloride. The œnanthylate disappears somewhat more slowly, although in Experiment 15 this is not the case. The caprylate is somewhat more rapidly absorbed than the sulphate.

The lactate (Experiments 11 and 13) seems to lie midway between the chloride and sulphate of soda.

5. *Oxalic acid series:* Oxalate, malonate, and succinate of sodium. Experiments 3, 7, 8, 13, 25. The oxalate is but little absorbed, and always induces congestion and inflammation, from which the loop does not soon recover. It therefore resembles the fluoride. The malonate and succinate solutions are scarcely absorbed, but do not cause inflammation, and the loop recovers after the solution is removed.

6. *Tartrate, citrate, and malate of sodium:* Experiments 3, 5, 6, 7, 8, 13, 14. The solutions of these three salts are absorbed at about the same rate as those of the sulphates. The first two are well-known saline cathartics.

7. *Salts of the aromatic acids:* Salicylate, ortho-phthalate, and para-phthalate of soda. Experiments 12, 17. The phthalates seem to disappear more slowly than the chlorides, but do not retard absorption to the same extent as do the sulphates. The salicylate was also slowly absorbed, but was used in only one experiment, which is insufficient to determine its exact position.

8. *The metallic ions:* Sodium, potassium, ammonium, magnesium, barium, calcium. In all the experiments except 23, 24, and 25 these are combined with $-SO_4$, $-NO_3$, $-FeCy_3$, and $-FeCy_4$ ions. In Experiments 23, 24, and 25, their chlorides and calcium acetate are compared. No difference could be detected in the behavior of the potassium and sodium salts. The ammonium chloride solution disappeared in Experiment 25 more rapidly than the standard sodium chloride solution, while in Experiment 24 it was absorbed at least as quickly; but here the sodium chloride solution was also entirely absorbed, so that it is impossible to state which was taken up the more rapidly from the bowel in this experiment. The salts of the alkaline earths were absorbed much more slowly than the corresponding salts of the alkalies. As regards the cations, therefore, our results are practically identical with those of Höber.

The soda salts can thus be arranged into four fairly distinct groups, according to the rate of absorption of their solutions.

TABLE I.

I.	II.	III.	IV.
Chloride. Bromide. Iodide.			Fluoride.
	Ethyl-Sulphate. Nitrate.	Sulphate. Phosphates.	
		Ferrocyanide. Ferricyanide.	
Formate. Acetate. Propionate. Butyrate. Valerate. Caproate.	Œnanthylate. Lactate.	Caprylate.	
		Malonate. Succinate.	Oxalate.
		Tartrate. Citrate. Malate.	
	Salicylate. Phthalates.		

The solutions of the salts of column I are all absorbed equally rapidly. Those of column II vary more or less in their behavior, but are generally absorbed more slowly than those of I. Those of III disappear very slowly, but, as a general rule, do not impair the absorption of the loop permanently, while the solutions of IV scarcely lessen at all in amount and evidently injure the loop seriously, for the solutions subsequently injected are only slowly absorbed or may even

increase in amount. We are not inclined to look upon this as differentiating the salts of IV from those of III qualitatively but only quantitatively, for the subsequent absorption was sometimes impaired by the salts of III, and Hay also found that strong solutions of sodium sulphate reduced the absorption of sodium chloride afterwards.

This table resembles in many features that given by Hofmeister to indicate the relative power of different salts to precipitate egg albumin.[1] His tables may be abbreviated by arranging the salts in two columns. It must be premised that the cations seem to have more influence here than in the intestine.

Ions with little or no power of precipitation.	*Ions with greater power of precipitation.*
Chlorides.	Sulphates.
Bromides.	Phosphates.
Iodides.	Acetates.
Nitrates.	Citrates.
Some acetates and chromates.	Tartrates.
	Chromates.

In experiments on the precipitation of gelatine with neutral salts he obtained similar results, and also in those on the precipitation of colloid iron oxide, while the sodium oleate solutions behave differently in regard to some salts.[2]

Hofmeister[3] found that gelatine plates absorbed less fluid when soaked in sulphate, tartrate, citrate, or acetate solutions than in chlorides, chlorates, nitrates, or bromides. Here, as in his experiments on the precipitation of egg albumin, the results are calculated for normal solutions so that they may be considered as isotonic except in so far as the dissociation of the salt is concerned. His experiments on the imbibition by pieces of the bladder wall were unfortunately carried out with percentage solutions and cannot be utilized for comparison. The same is true of Limbeck's[4] experiments on the diuretic action of salts.

It will be seen that Hofmeister's results do not quite correspond with ours, although they bear a very close resemblance. The most

[1] Hofmeister: Archiv für exper. Pathol. und Pharmakol., 1888, xxiv, p. 247.
[2] Hofmeister: *Ibid.*, 1888, xxv, p. 1.
[3] Hofmeister: *Ibid.*, 1891, xxviii, p. 210.
[4] Limbeck: Archiv für exper. Pathol. und Pharmakol., 1888, xxv, p. 69.

striking difference is in the behavior of some of the acetates, which precipitate proteids and other colloids and prevent the imbibition of gelatine plates much more than the chlorides do, while in our experiments solutions of the acetates and chlorides are equally rapidly absorbed from the intestine.

In spite of this and of some other minor differences which may be found by comparing Hofmeister's original tables with ours, there is a very striking similarity in our results, — most of those salts which precipitate egg albumin and prevent the permeation of gelatine plates also retard the absorption of fluid from the intestine. This would seem to support the view that these salts act as saline cathartics not through their lessening the "physiological activity" of the intestinal wall, as Heidenhain supposed, but through their being devoid of some general relation to colloid substances, organized or unorganized. This would not entail the belief that absorption from the intestine is a purely physical process, for the suggested explanation only covers the absorption of the fluid into the epithelium, and does not attempt to account for its transmission to the bloodvessels.

On the other hand some facts point to the opposite conclusion, namely, that the reaction of the intestinal epithelium to the salts is not due to the general physical properties of colloids. Thus Hedin[1] investigated the behavior of the red cells of the blood in solutions of various ammonium salts, and found that they were permeated without resistance by the chloride, bromide, sulpho-cyanate, oxalate, ferro- and ferricyanide, lactate, and ethyl-sulphate, while the sulphate, phosphate, tartrate, and succinate penetrated them with difficulty. These results present much greater contrasts to ours than Hofmeister's do, for while the ions that penetrate the red blood cells with difficulty also prevent the absorption of fluids by the intestinal wall, several ions that permeate the blood corpuscles with ease act as cathartics (oxalates, ferrocyanides), and others stand midway between the cathartics and the indifferent ions (ethyl-sulphate, lactate). It is evident therefore that the colloids of the red blood cells and those of the intestinal epithelium differ very considerably in their relations to different anions, although there are some common features. This conclusion is confirmed by the fact that the red cells are permeated only with the greatest difficulty by the fixed alkali ions, whereas comparatively little resistance is offered by the intestine.

[1] Hedin: *loc. cit.*

Again, Leathes and Starling[1] found that the pleural endothelium absorbed solutions of magnesium sulphate and sodium sulphate as rapidly as those of sodium chloride, so that here the cell contents present yet another variation in their affinities.

Lastly Pohl,[2] Young,[3] and others have investigated the precipitation of colloid carbohydrates by neutral salts and find a considerable variation in their relations. Pohl states that the sulphate of ammonia precipitates a larger number of these than the phosphate of ammonia or the acetate of potash, while these again act on a larger number than the sulphate of magnesium. The conclusion seems inevitable that while a general resemblance may exist in the relation of the neutral salts to the different groups of colloid bodies, the details vary with each individual colloid. This differentiation of salts into two series, — the one permeating the intestinal epithelium, the other apparently repelled by it, naturally demands explanation, and we have therefore attempted to find some further characters common to the cathartic salts and not possessed by the indifferent salts.

Loeb[4] has recently advanced the view that the action of some substances may be determined by the number of dissociated ions, and by their velocity. The amount of dissociation can scarcely be expected to have much importance, however, where identical effects are obtained with two salts which vary so greatly in their dissociation as the chloride and acetate of sodium. Dr. K. Guthe of the physical laboratory had the kindness to ascertain the relative electrolytic conductivity of our solutions, and we found that it bore no relation to their behavior in the intestine. For example, the sodium chloride solution gave a deviation of the electrometer of 158, the acetate of 97.5, the fluoride of 110, the oxalate of 155, the tartrate of 137, and the sulphate of 235. The purgative fluoride and oxalate therefore stand between the indifferent acetate and chloride. The variation in the velocity of the anions is also apparently without significance, for as it decreases with an increase in the atomic weight the purgative caprylate ion must have a smaller velocity than the indifferent caproate or acetate, while the oxalate ion on the other hand must have a greater velocity than the succinate, which however is less purgative.

[1] Leathes and Starling: Journal of physiology, 1895, xviii, p. 136.

[2] Pohl: Zeitschr. f. physiol. Chemie, 1890, xiv, p. 151.

[3] Young: Journal of physiology, 1897, xxi, p. xvi.

[4] Loeb: Arch. f. d. ges. Physiol., 1897, lxix, p. 1.

The physical differences of the solutions do not present any relation to the differences in their action, then, and we have sought for some pharmacological property common to the cathartics, and not possessed by the indifferent salts. As regards the oxalate and fluoride (4th column, table I), this might be found in their action as general protoplasmic poisons; the connection between this and their action on the bowel is rendered more plausible by the fact that the addition of quinine hydrochlorate, a well known protoplasmic poison, to the standard solution prevents absorption and causes congestion and irritation, although the quantity added is too small to alter the osmotic pressure.

It is more difficult to find any relation between the substances of the third column, for while the tartrate and citrate are undoubtedly poisonous when injected into the blood, the sulphate has little or no such effect. Most of these salts are dibasic or tribasic, while those of the first column are monobasic; but the significance of this fact is lessened by the presence of the phthalates in the second column, and of the caprylates in the third.

The lower members of the acetic acid series permeate freely, but a sudden change occurs when the œnanthylate and caprylate are reached. This would suggest that the increasing size of the molecule influenced the rate of absorption, but this does not hold good in other cases, for the malonate and succinate — the higher members of the oxalic acid series — are less cathartic than the lowest homologue, the oxalate.

The second column is even less homogenous than the third. The œnanthylate may be looked upon as bridging the gap between the permeating simpler members and the purgative higher members of the acetic acid series, while the lactate and salicylate bear some relation to each other in both being oxy-acids. The phthalate and ethyl-sulphate, on the other hand, might have been expected in the third column, for the former is a dibasic salt, like most of the other salts of the third column, while the ethyl-sulphate might be expected to resemble the simple sulphate.

One curious relation, which struck us early in our experiments, and which determined to some extent the direction of our work, was that existing between the behavior of the ions in the intestine and the solubility of the corresponding calcium salts.

The solubility of some of these salts has not been determined, and we have therefore ascertained them by shaking the calcium salt in

water for three or four hours and estimating the amount of the salt dissolved in a given quantity of the filtered solution by evaporating and weighing. The results of the estimations made by others and by ourselves are given in the following table, in which the figures in the first column give the number of grams of salt dissolved in 100 c.c. water, while the figures in the second column give the temperature at which the estimation was made. We have selected temperatures at 40° C. where possible, so as to approach the conditions in the body more nearly.

TABLE II.

Grams of Calcium Salt dissolved in 100 c.c. of water at the temperature given.

	Grams	°C.		Grams	°C.
Calcium iodide[5]	228.00	40.0	Calcium œnanthylate[7]	0.786	40.0
bromide[5]	213.00	40.0	malate	0.753	21.5
chloride[5]	104.00	35.0	ferrocyanide	0.580	23.0
nitrate[5]	82.40	....	phthalate (ortho)	0.528	21.5
propionate[4]	37.72	40.0	malonate[2]	0.422	40.0
acetate[3]	33.90	40.0	sulphate[1]	0.210	38.0
formate[3]	17.40	39.7	caprylate	0.133	21.5
butyrate[6]	16.30	40.0	citrate	0.089	21.5
lactate	....	....	tartrate	0.045	21.5
valerate[4]	8.20	40.0	hydric phosphate[8]	0.028	0.0
caproate[3]	2.50	40.0	fluoride[8]	0.017	15.0
succinate[2]	1.15	41.6	oxalate	0.0	21.5

[1] Raupenstrauch: Monatshefte für Chemie, 1885, vi, p. 570.
[2] Miczynski: *ibid.*, 1886, vii, p. 255.
[3] Krasnicki: *ibid.*, 1887, viii, p. 505.
[4] Fürth: *ibid.*, 1888, ix, p. 308.
[5] Keppich: *ibid.*, 1888, ix, p. 380.
[6] Dezathy: *ibid.*, 1893, xiv, p. 250.
[7] Landau: *ibid.*, 1893, xiv, p. 707.
[8] Comey: Dictionary of chemical solubilities, London, 1896.

On comparing Table I and Table II, it will be observed at once that the most soluble calcium salts are those formed by combinations with the indifferent ions (first column, table I), while the cathartic salts of the third column form very much less soluble salts with

calcium and the fluoride and oxalate (fourth column) are entirely insoluble. This is remarkably exemplified by the behavior of the acetic series, for while the first six members of this series are indifferent in the intestine and form fairly soluble salts with lime, the seventh (œnanthylic) is slowly absorbed and rather insoluble, and the eighth (caprylic), which is very insoluble, acts in the same way as the sulphates. In the same way the least permeating member of the oxalic acid series (oxalic) forms an absolutely insoluble lime salt, while the less cathartic higher members form more soluble compounds with calcium.

Some exceptions to the general rule undoubtedly exist, apart from the nitrates, which we do not regard as of the same class as the others. Thus the succinate of calcium is more soluble than the œnanthylate, and yet sodium succinate is more cathartic, while the phthalates are less soluble[1] and yet appear in the second column. The lactate and salicylate also form rather soluble lime salts and yet appear to be somewhat slowly absorbed. Another exception is the ethyl-sulphate, which forms a very soluble lime salt, but it seems not impossible that this body may in part be decomposed in the course of absorption, in which case the sulphate formed would retard absorption. Similarly, the ferricyanide of calcium is soluble, but the sodium salt is reduced to the ferrocyanide in the intestine and therefore retards absorption.

It is to be remarked, however, that no very soluble lime salt is formed by the really cathartic group of ions (third and fourth columns, table I), while no acid forming insoluble lime salts is found in the first column. The exceptions cited above all fall into the second column, which is a makeshift group of substances neither entirely indifferent nor sufficiently slowly absorbed to entitle them to a place among the distinctly cathartic salts. Besides, it is very evident that the property which prevents the absorption of certain ions, and at the same time renders their combinations with lime insoluble, is not the only determining factor in absorption, for quinine hydrochlorate in traces prevents absorption. These exceptions therefore do not seem to us to invalidate the general result, namely, that acids which form insoluble salts with calcium act as

[1] The phthalates of calcium are said to differ considerably in solubility, but we found that the two phthalates precipitate lime water in the same degree of dilution. The quantity at our disposal did not admit of more accurate chemical examination.

cathartics when combined with ordinarily indifferent bases such as the alkalies.

The question at once arises whether the connection between these two properties is a causal one, *i. e.*, whether the cathartic salts are slowly absorbed because they precipitate calcium in the intestinal wall. It is needless to say that this is a possible explanation, for the precipitation of calcium has been shown to have a very considerable effect in such processes as the coagulation of the blood and of milk. The importance of calcium in the nutrition of the heart and of developing ova (Ringer), in the contraction of muscle (Locke), in the irritability of nerve fibres (Howell), and in the growth of plants (Loew) is generally recognized,[1] and we are tempted to suppose that in the absorption from the bowel the calcium plays a similar rôle. We feel however that our experiments are not sufficient to allow of a positive statement, and must leave the question open for the present. Howell's work on the action of oxalates on the heart left him in the same position of uncertainty as to whether the effects were due to a precipitation of calcium or to some specific action of the oxalates.[1] In this relation we may mention that in a number of experiments which we have performed on the tortoise heart the sulphate of sodium seemed to have the same effect as the acetate, while the citrate was extremely poisonous.

In the account of our results hitherto we have tacitly assumed that the salt failed to permeate the intestinal wall. This assumption is based upon results obtained by Hay, and more lately by Kovesi[2] and confirmed by our own observations that a considerable amount of the cathartic salt remains in the fluid in the intestine. Some salt undoubtedly disappears, but not nearly so much as when solutions of chloride of sodium or of any other indifferent salt are used. The depression of the freezing point ($\triangle$) of the residue remains unchanged if the solution was originally isotonic, as were most of our solutions. If on the other hand a hyperisotonic solution is injected, the $\triangle$ slowly declines to about .61 (that of the blood), while if a hypistonic solution is used, a concentration of the fluid sets in until the $\triangle$ again approaches that of the blood. This is in accord with Kovesi's results on the rabbit's intestine, but does not conflict as he supposes with Heidenhain's results obtained with sodium chloride

[1] Howell: Journal of physiology, 1894, xvi, p. 476.

[2] Kovesi: Centralblatt fur Physiologie, 1897, xi, p. 553.

solutions, for the alteration in the △ of the intestinal contents is evidently due in both cases to the osmotic interchange of fluid and salt with the blood, which Heidenhain fully recognized.

In many of our experiments a considerable amount of mucus was present in the residual fluid, but this was not constant, and there did not seem more mucus in the residue of the cathartic solutions than in that of the standard solution. In many of the intestinal loops tapeworms were present, and in these there seemed more mucus than elsewhere. Hay[1] is inclined to look upon the increased secretion of mucus by the intestine under sodium sulphate as of some importance in retarding absorption, and this explanation has been again brought forward by Fusari and Marfori.[2] We are not disposed to look upon the secretion of mucus as of much importance in determining the absorption or non-absorption of the cathartic solutions.

The objection may always be brought against the method we have adopted that the conditions are so abnormal that no inferences as to the behavior of the uninjured intestine can be drawn. On the other hand no accurate results can be obtained by measuring the fluid in the fæces after the use of one of the purges, because the amount of fluid in the bowel previously is unknown. We have therefore attempted to determine the action of these purgatives by comparing the amount of fluid which escaped from a cæcal fistula after the administration by the stomach of isotonic solutions of various salts.

A medium sized dog was chloroformed, the abdomen laid open, and a loop of intestine immediately above the termination of the ileum sewed into the wound. Four days later, when complete adhesion had occurred, and the wound was rapidly healing, the loop was opened. A week later, the examination of the action of different salts was commenced. The animal received no food in the morning and in the afternoon a measured quantity of sodium chloride (△ .615) was administered by the stomach tube and the amount of fluid passed by the fistula during the next hour measured. When no more fluid was passed an equal amount of an isotonic solution of another salt was given in the same way, and the fluid escaping by the fistula again measured. The results confirmed those obtained by the other method, but the investigation could not be carried far as the animal died, apparently from having been exposed to great cold during the night of Dec. 25.

[1] Hay: Saline cathartics, 1884, p. 69.

[2] Fusari and Marfori: Atti della acad. delle scienze med. e nat. in Ferrara, 1894; cited from Centralbl. f. innere Medicin, 1894, p. 1245.

Our results were as follows:

Dec. 21, 1897.

Experiment 1. Injected into stomach 100 c.c. NaCl Δ .615.
15′ Some solid matter evacuated with a few c.c. fluid.
60′ Total amount discharged 5 c.c. fluid and fæcal matter.

Experiment 2. Injected 100 c.c. sodium citrate Δ .62.
15′ Some fæcal matter discharged and fluid began to appear.
60′ Total amount of fluid discharged = 70 c.c.

Dec. 22.

Experiment 1. Injected 100 c.c. NaCl Δ .615.
60′ Total amount discharged = 4 c.c.

Experiment 2. Injected 100 c.c. sodium acetate Δ .615.
60′ Total amount discharged = 0.

Experiment 3. Injected 100 c.c. sodium phthalate (ortho) Δ .62.
60′ Total amount discharged = 0.

Dec. 23.

Experiment 1. Injected 100 c.c. NaCl Δ .615.
60′ Total amount discharged = 0.

Experiment 2. Injected 80 c.c. { sodium phthalate (ortho) Δ .62. / sodium phthalate (para) Δ .56.
60′ Total amount discharged = 0.

Dec. 24.

Experiment 1. Injected 100 c.c. NaCl Δ .615.
60′ Total amount discharged = 0.

Experiment 2. Injected 100 c.c. Na_2SO_4 Δ .62.
60′ Total amount discharged = 75 c.c.

The whole of the solutions of sodium chloride, sodium acetate, and sodium phthalate was absorbed in the course of its passage through the stomach and small intestine, while three fourths of the citrate and sulphate solutions reached the large intestine, and in the normal animal would have gone to increase the fluidity of its contents. We think that this demonstrates conclusively the method of action of the dilute solutions of the cathartics such as are found in some of the natural mineral waters. They do not necessarily increase the fluid of the bowel, but merely fail to be absorbed, and thus render the fæces more fluid and more easily moved through the large intestine.

Conclusions.

1. The absorption of the salts of the fixed alkalies varies with the anion, those acids which form insoluble calcium salts tending to retard absorption more than others.

2. The behavior of these salts in the intestine has much in common with their action on unorganized colloid matter, as they tend to precipitate colloids in solution and are less imbibed than other salts by undissolved colloids.

3. But no complete analogy in their behavior towards the tissues in general exists, for several of the cathartic salts permeate the red corpuscles freely and others are absorbed rapidly from the serous membranes.

4. As regards the cations, ammonium is absorbed more rapidly than the fixed alkali ions, while those of the alkaline earths are very slowly taken up by the intestinal epithelium.

5. Dilute solutions (isotonic) of the saline cathartics retard the absorption of fluid from the stomach and small intestine, and thus act by rendering the contents more watery and more easily moved through the lower parts of the alimentary canal.

Protocols of the experiments are given on pages 429–434.

	Time in minutes	Loop I.			Loop II.			Loop III.			Remarks
		Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue	
Experiment 1 — Nov. 1, 1897 — **Cat.**											
a	30	NaCl	.59	3.0 c.c.	$MgSO_4$	.62	15.5 c.c.	....	....	....	Length of each loop = 45 cm. Amount of solution injected into each loop = 15 c.c.
b	30	NaCl	.59	5.5	$MgSO_4$	.62	15.0	....	....	....	
c	60	NaCl	.59	6.0	$MgSO_4$	.62	25.0	....	....	....	
Experiment 2 — Nov. 2, 1897 — **Cat.**											
a	30	NaCl	.59	4.0	$MgSO_4$	.62	9.0	....	....	....	Length of each loop = 45 cm. Amount of solution injected = 15 c.c.
b	30	NaCl	.59	8.0	Na_2SO_4	.62	14.0	....	....	....	
c	60	NaCl	.59	7.5	Na_2SO_4	.62	14.0	....	....	....	
Experiment 3 — Nov. 4, 1897 — **Cat.**											
a	30	NaCl	.59	3.0	NaOxalate	.625	9.5	....	....	....	Length of each loop = 45 cm. Amount of solution injected = 15 c.c.
b	40	NaOxalate	.625	17.0	NaCl	.59	13.0	....	....	....	
c	30	NaTartrate	.625	27.0	NaCl	.59	19.5	....	....	....	
Experiment 4 — Nov. 8, 1897 — **Cat.**											
a	30	NaAcetate	.62	8.5	NaCl	.59	10.0	....	....	....	Length of each loop = 45 cm. Amount of solution injected = 15 c.c.
b	30	NaCl	.59	11.5	NaAcetate	.62	10.0	....	....	....	
Experiment 5 — Nov. 9, 1897 — **Cat.**											
a	30	NaCl	.59	0.0	NaTartrate	.625	9.0	....	....	....	Length of each loop = 45 cm. Amount of solution injected = 15 c.c.
b	30	NaTartrate	.625	10.0	NaCl	.59	10.5	....	....	....	

	Time in minutes	Loop I.			Loop II.			Loop III.			Remarks
		Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue	
Experiment 6 — Nov. 10, 1897 — **Cat.**											
a	30	NaCl	.59°	3.5 c.c.	NaCitrate	.61°	13.5 c.c.	....	....	....	Length of each loop = 45 cm.
b	30	NaCl	.59	6.5	NaCitrate	.54	16.0	....	....	....	Amount injected = 15 c.c.
c	30	NaCitrate	.61	15.0	NaCl	.59	16.0	....	....	....	Loops washed with normal salt solution previous to injection.
Experiment 7 — Nov. 11, 1897 — **Dog.**											
a	30	NaCl	.59	2.0	NaCl	.59	2.0	NaCl	.59°	7.0 c.c.	Length of { Loop I. = 42.5 cm. Loop II. = 37.5 cm. Loop III. = 35 cm.
b	30	NaCl	.59	2.0	NaOxalate	.625	22.0	NaAcetate	.62	2.0	
c	30	NaCl	.59	2.9	NaOxalate	.625	40.0	NaAcetate	.62	3.0	
d	30	NaOxalate	.625	22.0	NaCl	.59	21.5	NaTartrate	.625	17.5	Amount injected = 25 c.c.
											Loops washed with normal salt solution.
Experiment 8 — Nov. 12, 1897 — **Dog**											
a	30	NaCl	.615	2.0	NaCl	.615	4.0	NaCl	.615	7.0	Length of each loop = 30 cm.
b	30	NaCl	.615	7.5	NaMalate	.635	14.5	NaMalonate	.575	23.0	Amount injected = 25 c.c.
c	30	NaCl	.615	9.0	NaMalate	.635	19.0	NaMalonate	.575	23.0	Loops washed with normal salt solution.
Experiment 9 — Nov. 18, 1897 — **Dog.**											
a	30	NaCl	.615	1.0	NaCl	.615	3.0	NaCl	.615	10.5	Length of each loop = 30 cm.
b	30	NaCl	.615	2.0	NaCl	.615	9.0	NaCl	.615	12.0	Amount injected = 25 c.c.
c	30	NaCl	.615	7.0	Na_2HPO_4	.56	13.0	NaH_2PO_4	.56	22.0	Loops washed with normal salt solution.
d	30	Na_2HPO_4	.56	19.0	....	....	....	....	....	....	

	Time in minutes	Loop I			Loop II			Loop III			Remarks
		Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue	
Experiment 10 — Nov. 19, 1897 — **Cat.**											
a	30	NaCl	.615°	2.0 c.c.	NaCl	.615°	4.0 c.c.	....	....	....	Length of each loop = 45 cm.
b	30	NaH_2PO_4	.56	7.0	Na_2HPO_4	.56	8.0	....	....	....	Amount injected = 15 c.c.
c	30	NaCl	.615	8.0	Na_2HPO_4	.56	15.5	....	....	....	Loops washed with normal salt solution
Experiment 11 — Nov. 29, 1897 — **Dog.**											
a	30	NaAcetate	.615	1.5	NaFormate	.63	4.0	NaPropionate	.66°	7.0 c.c.	Length of each loop = 30 cm.
b	30	NaAcetate	.62	1.5	NaButyrate	.52	2.0	NaValerate	.69	7.0	Amount injected = 25 c.c.
c	30	NaCaprylate	.61	24.0	NaCaproate	.62	4.0	NaAcetate	.615	10.0	
d	35	NaLactate	.62	15.0	NaLactate	.62	7.0	NaAcetate	.615	3.0	
Experiment 12 — Dec. 1, 1897 — **Dog.**											
a	30	NaCl	.615	6.0	NaCl	.615	2.75	NaCl	.615	1.0	Length of each loop = 30 cm.
b	30	NaCl	.615	13.0	NaPhthalate (ortho)	.62	10.5	NaPhthalate (para)	.56	7.0	Amount injected = 25 c.c.
c	30	NaCl	.615	9.0	NaCl	.615	5.0	NaCl	.615	2.5	Loops washed with normal salt solution.
d	30	NaCl	.615	8.0	NaPhthalate (ortho)	.62	12.0	NaPhthalate (para)	.56	11.0	
Experiment 13 — Dec. 3, 1897 — **Dog.**											
a	30	NaCl	.615	15.0	NaCl	.615	13.0	NaCl	.615	16.0	Length of each loop = 30 cm.
b	30	NaCl	.615	17.0	NaPropionate	.66	14.5	NaValerate	.625	11.5	Amount injected = 25 c.c.
c	30	NaCl	.615	16.0	NaButyrate	.52	7.0	NaTartrate	.625	21.0	

	Time in minutes	Loop I.			Loop II.			Loop III.			Loop IV.		
		Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue
Experiment 14 — Dec. 4, 1897 — **Dog**.													
a	30	NaCl	.615°	0.5 c.c.	NaCl	.615°	5.0 c.c.	NaCl	.615°	0.5 c.c.	NaCl	.615°	1.5 c.c.
b	30	NaCl	.615	0.5	NaMalate	.635	13.0	NaMalonate	.575	18.5	NaCitrate	.62	19.0
c	30	NaCl	.615	1.0	NaCl	.615	5.5	NaCl	.615	7.0	NaCl	.615	10.0
d	30	NaLactate	.62	14.0	NaCaprylate	.61	14.5	NaPropionate	.66	12.0	NaCl	.615	10.0
Remarks. — Length of each loop = 30 cm. Amount injected = 25 c.c.													
Experiment 15 — Feb. 7, 1898 — **Dog**.													
a	30	NaCl	.64	1.25	NaCl	.64	1.75	....	....	....	....	....	....
b	30	NaCaproate	.61	1.75	NaEnanthylate	.625	1.75	....	....	....	....	....	....
c	30	NaCl	.64	8.0	NaCl	.64	1.0	....	....	....	....	....	....
d	30	....	....	....	NaCaprylate	.60	7.0	....	....	....	....	....	....
Remarks. — Length of each loop = 45 cm. Amount injected = 25 c.c.													
Experiment 16 — Feb. 9, 1898 — **Dog**.													
a	30	NaCl	.61	0.0	NaCl	.61	0.0	NaCl	.61	0.0	NaCl	.61	0.0
b	30	NaCl	.61	8.0	NaCaproate	.61	5.0	NaEnanthylate	.625	10.5	NaCaprylate	.60	15.0
c	30	NaCl	.61	6.5	NaCl	.61	7.0	NaCl	.61	5.0	NaCl	.61	7.0
d	30	NaCl	.61	8.0	NaCaproate	.61	5.0	NaEnanthylate	.625	9.0	NaCaprylate	.60	15.5
Remarks. — Length of each loop = 30 cm. Amount injected = 25 c.c.													
Experiment 17 — Feb. 10, 1898 — **Dog**.													
a	30	NaCl	.61	1.5	NaCl	.61	3.0	NaCl	.61	1.0	NaCl	.61	4.5
b	30	NaCl	.61	5.0	NaCaproate	.61	3.0	NaEnanthylate	.625	8.5	NaCaprylate	.60	15.5
c	30	NaCl	.61	5.0	NaCl	.61	4.0	NaCl	.61	4.0	NaCl	.61	15.0
d	30	NaCl	.61	7.0	NaI	.62	3.0	....	....	....	....	....	....
Remarks. — Length of each loop = 30 cm. Amount injected = 25 c.c.													

	Time in minutes	Loop I.			Loop II.			Loop III.			Loop IV.		
		Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue
Experiment 18 — Feb. 10, 1898 — **Dog.**													
a	30	NaCl	.64°	7.25 c.c.	NaCl	.64°	3.25 c.c.	NaCl	.64°	5.0 c.c.	NaCl	.61°	2.0 c.c.
b	30	NaCl	.64	9.0	$NaNO_3$	.65	15.0	KNO_3	.63	14.5	NaI	.62	9.0
REMARKS. — Length of each loop = 30 cm. Amount injected = 25 c.c.													
Experiment 19 — Feb. 15, 1898 — **Dog.**													
a	30	NaCl	.64	3.0	NaCl	.64	2.5	NaCl	.64	5.0	NaCl	.64	2.5
b	30	NaCl	.64	3.0	NaBr	.60	5.5	NaFl	.60	20.0	NaI	.62	15.0
c	30	NaI	.62	8.0	NaCl	.64	2.5	NaCl	.64	26.0	NaCl	.64	2.5
d	30	NaCl	.64	5.0	NaSalicylate	.62	9.0	NaCl	.64	9.0	NaCl	.64	2.5
REMARKS. — Length of each loop = 30 cm. Amount injected = 25 c.c.													
Experiment 20 — March 1, 1898 — **Dog.**													
a	30	NaCl	.64	8.0	NaCl	.64	8.5	NaCl	.64	9.5	NaCl	.64	9.5
b	30	NaCl	.64	8.0	$KC_2H_5SO_4$	.63	18.0	Na_2SO_4	.60	27.5	$(NH_4)_2SO_4$	.58	21.0
c	30	NaCl	.64	8.0	NaCl	.64	19.0	NaCl	.64	6.0	NaCl	.64	6.0
d	30	NaCl	.64	5.5	....	....	....	NaCl + Quinine HCl	....	20.0	NaCl + Quinine HCl	....	19.0
REMARKS. — Length of each loop = 30 cm. Amount injected = 25 c.c. $KC_2H_5SO_4$ contains free SO_4.													
Experiment 21 — March 15, 1898 — **Dog.**													
a	30	NaCl	.625	3.0	NaCl	.625	1.0	NaCl	.625	1.0	NaCl	.625	2.0
b	30	NaCl	.625	4.5	$NaC_2H_5SO_4$	.62	8.0	Na_2SO_4	.835	50.0	Na_2SO_4	.835	45.0
c	30	NaCl	.625	4.0	NaCl	.625	1.5	NaCl	.625	7.0	NaCl	.625	5.5
d	30	KFerrocyanide	.63	27.0	KFerricyan	.63	22.0	NaCl	.625	5.5	$NaC_2H_5SO_4$	.62	23.0
REMARKS. — Length of each loop = 40 cm. Amount injected = 30 c.c.													

	Time in minutes	Loop I.			Loop II.			Loop III.			Loop IV.		
		Salt	Δ	Residue	Salt.	Δ	Residue	Salt	Δ	Residue	Salt	Δ	Residue
Experiment 22 — March 21, 1898 — **Dog.**													
a	30	NaCl	.625°	10.5 c.c.	NaCl	.625°	13.0 c.c.	NaCl	.625°	1.0 c.c.	NaCl	.625°	2.0 c.c.
b	30	NaCl	.625	6.5	Na_2SO_4	.625	30.0	$Na(C_2H_5)SO_4$	.62	13.5	Na_2SO_4	.625	25.0
c	30	NaCl	.625	10.5	NaCl	.625	17.0	NaCl	.625	7.75	NaCl	.625	5.0
d	30	KFerrocyan.	.63	33.0	NaCl	.625	11.5	KFerricyan.	.63	25.5	$Na(C_2H_5)SO_4$	.62	17.0
REMARKS. — Length of each loop = 45 cm. Amount injected = 35 c.c.													
Experiment 23 — March 22, 1898 — **Dog.**													
a	30	NaCl	.625	0.0	NaCl	.625	1.0	NaCl	.625	1.5	NaCl	.625	1.0
b	30	NaCl	.625	3.0	$MgCl_2$	.645	29.0	$MgSO_4$	.25	22.0	NaFerrocyan.	.63	31.5
c	30	NaCl	.625	5.0	NaCl	.625	4.5	NaCl	.625	5.0	NaCl	.625	4.5
d	30	KFerrocyan.	.37	22.5	$MgCl_2$	.645	28.5	$MgSO_4$	.25	19.5	$MgSO_4$	.52	26.0
REMARKS. — Length of each loop = 45 cm. Amount injected = 30 c.c.													
Experiment 24 — March 29, 1898 — **Dog.**													
a	30	NaCl	.625	1.75	NaCl	.625	0.0	NaCl	.625	5.5	....	....	....
b	30	KCl	.625	0.0	NH_4Cl	.60	1.0	NaCl	.625	3.5	....	....	....
c	30	BaCl	.62	18.0	CaAcetate	.62	16.0	$MgCl_2$	.645	18.0	....	....	....
d	30	NaCl	.625	7.5	NaCl	.625	1.0	NaCl	.625	3.0	....	....	....
e	30	$NaNO_3$	.65	12.5	KNO_3	.63	21.0	NaCl	.625	6.5	....	....	....
REMARKS. — Length of Loop I = 45 cm.; Loop II = 45 cm.; Loop III = 30 cm. Amount injected = 30 c.c.													
Experiment 25 — April 18, 1898 — **Dog.**													
a	30	NaCl	.625	0.0	NaCl	.625	1.0	NaCl	.625	0.0	NaCl	.625	8.5
b	30	NaOxalate	.625	30.0	NaMalonate	.625	15.5	NaSuccinate	.62	17.0	NaCl	.625	9.5
c	30	NaCl	.625	26.5	NaCl	.625	7.0	NaCl	.625	0.0	NaCl	.625	4.5
d	30	NaCl	.625	22.0	NaCl	.625	5.0	NaMalonate	.625	18.0	NaSuccinate	.62	22.0
e	15	....	....	....	NH_4Cl	.60	16.0	NaCl	.625	22.5	NH_4Cl	.60	15.0
REMARKS. — Length of each loop = 45 cm. Amount injected = 30 c.c.													

THE MOVEMENTS OF THE FOOD IN THE ŒSOPHAGUS.

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THE movements of deglutition, in common with many other physiological processes, were explained by the older physiologists on anatomical grounds. Thus Magendie[1] divided the act into three parts, corresponding to the anatomical regions of the mouth, pharynx, and œsophagus. The muscles of each of these divisions were considered the active agents in propelling the food onward. The function of moving the mass to the pharynx was variously ascribed to the tongue itself, to the mylohyoid muscles, and to gravity. For the second part, the movement through the pharynx, there was more unanimity of opinion, since the constrictors, especially the middle and lower, were evidently concerned.

Direct observations on the movement of swallowed masses in the œsophagus were first made by Mosso[2] The œsophagus of a dog was laid bare and a transverse incision made through it, or a piece of it excised. A small wooden ball was placed in the canal below the excised part, and the animal was then stimulated to swallow. One or two seconds after the contraction of the pharyngeal muscles a peristaltic wave began to traverse the œsophagus. This wave did not stop at the point of excision, but in due time reappeared below and carried the ball to the stomach. Thus the act was shown to be controlled by the central nervous system. Peristalsis was so plainly the motive power that the action was never doubted. Yet this belief was soon to be questioned.

In 1880, Falk and Kronecker[3] studied the movements in the mouth and pharynx, and advanced the theory that deglutition was accomplished by the rapid contraction of the muscles of the mouth. During the act of swallowing the air-tight buccal cavity shows a manometric pressure of 20 centimetres of water. The same pressure was demonstrated to be present also in the œsophagus, but not in

[1] Magendie: Précis élémentaire de physiologie. Paris, 1836, i, p. 63.

[2] Mosso: Moleschott's Untersuchungen, 1876, xi, p. 331.

[3] Falk and Kronecker: Archiv für Physiologie, 1880, p. 296.

the stomach. This pressure was considered sufficient to force food through the œsophagus before the peristaltic wave traversed it. Another argument for rapid descent was found in the fact that cold water can be felt in the epigastric region almost immediately after being swallowed. Further, when strong acids pass through the gullet, they corrode but small parts of it, and not the entire mucous membrane, as would be the case were the acid carried to the stomach by peristalsis.

In the same year, in confirmation of the above results the well known experiments of Kronecker and Meltzer[1] were published. A rubber balloon, connected by a tube to a Marey tambour, was placed in the pharynx, and another balloon, similarly connected, was introduced a varying distance into the œsophagus. When water was swallowed the increased pressure in the pharynx was transmitted to the first tambour, which traced a curve on a rotating drum. Almost instantly thereafter the œsophageal balloon was compressed, causing the second tambour to write its curve below the first. This second curve was supposed to mark the passage of the food through the œsophagus. After a varying number of seconds the œsophageal balloon recorded another curve, caused by a peristaltic wave which carried to the stomach any fragments left in the canal.

To demonstrate that the first curve of the œsophageal balloon was caused by the passage of the swallowed liquid, Meltzer devised another experiment. A strip of blue litmus paper was placed in a stomach tube, opposite the side openings at the lower end. A long thread attached to the paper ran through the tube to the other end. The tube was now passed into the lower end of the œsophagus and an acid drink swallowed. If the litmus paper was pulled away from the side openings a second after the beginning of swallowing, it was found distinctly reddened, showing a rapid descent of the swallowed liquid. Reference to this experiment will be made later.

From these observations Kronecker and Meltzer concluded that liquids and semi-solids are not carried to the stomach by peristalsis, but are squirted down the œsophagus by the rapid contraction of the muscles of the mouth. For this purpose the mylohyoids alone are sufficient, since the middle and inferior constrictors can be cut without interfering with the act. The succeeding peristalsis is of use merely in gathering up adhering fragments and carrying them to the stomach.

[1] Kronecker and Meltzer: Archiv für Physiologie, 1880, p. 446.

To determine whether the cardia offered any resistance to this rapid passage into the stomach, Meltzer[1] tried another method. If a stethoscope is placed over the epigastrium during the swallowing of liquids, a sound can be heard from six to seven seconds after the rise of the larynx. The sound is caused by the passage of the swallowed mass, liquid and air, through the tonically contracted cardia. In a few cases the sound is heard immediately after swallowing, showing a probable insufficiency of the cardia. These phenomena led Kronecker and Meltzer[2] to modify their previous views. They now maintained that the mass is not squirted by the mylohyoids directly into the stomach, but halts a short distance above the cardia. Here it remains until carried into the stomach by the succeeding peristalsis, about six or seven seconds after the beginning of swallowing.

The care with which these experiments were conducted has won general assent to their results. But the methods employed are not beyond criticism. Primarily it may be said that swallowing with one or more balloons and a stomach tube in the canal is not normal deglutition. Moreover, semi-solids were found to yield less readily to pressure than liquids, and even to be delayed in their descent.[3] Again nearly all the work was done with liquids and semi-solids; solids are not even mentioned. The investigators themselves declared that their results were true for liquids and semi-solids only, and admitted that a dry bolus could not be so swallowed. Yet the indiscriminate use of such terms as "liquid," "swallowed mass," and "bolus," easily leads to an inference that the results of these investigations are true for the swallowing of food of all consistencies. A difference in rate, however, certainly exists in respect to consistency, and it was to discover the actual movement of solids, semi-solids, and liquids in the normal œsophagus that the present work was undertaken.

Over a year and a half ago it was suggested by Prof. H. P. Bowditch that if some substance opaque to the Röntgen rays were swallowed, it could be seen in its passage to the stomach and the nature of its movement thus determined. Anæsthesia could be dispensed with, — a desirable condition, since observers had found that it inter-

[1] Meltzer: Centralbl. für die med. Wissenschaften, 1883, p. 1.

[2] Kronecker and Meltzer: Archiv für Physiologie, 1883, Suppl. Bd., p. 337, 351.

[3] Kronecker and Meltzer: *ibid.*, p. 337.

fered greatly with the deglutition reflex.[1] It would be unnecessary to open either the abdominal or the pleural cavity. The reflex stimulus of food moreover would be better than electrical stimulation of the superior laryngeal nerve. In short, the animal would swallow normal food under practically normal conditions. At Dr. Bowditch's suggestion and with his valuable assistance — which we gratefully acknowledge — we made the following series of experiments.

To render the swallowed mass opaque subnitrate of bismuth was used. The salt is tasteless, practically inert, and can be fed in large quantities without harm. In order that observations could be made by more than one person, all experiments were conducted in a dark room. On the side of the animal opposite the Crookes tube was placed an open fluorescent screen on which the different tissues of the animal were outlined with varying degrees of light and shade. Among these shadows the swallowed mass appeared as a darker object, and thus its motion could be studied.

For the first experiments the goose was selected. The head and neck were held stationary by a tall pasteboard collar which allowed free movement of the head without constriction of the neck. The fluorescent screen was placed against this collar at a uniform distance of thirty centimetres from the tube. When a bolus of corn meal mush mixed with bismuth was placed in the pharynx it descended slowly and regularly, and occupied about twelve seconds in passing over a distance of fifteen centimetres. The screen was marked at intervals of two centimetres with cross lines, by means of which the relative rate in different parts of the œsophagus could be studied. A vibrator marking tenths of a second was interrupted whenever the bolus crossed a line. An average of over one hundred such observations showed that the rate became slightly slower as the bolus proceeded.

In order to test liquids, molasses was mixed with bismuth to such a consistency as to drop easily from a glass rod. When this was fed with a pipette it passed slowly and regularly down the œsophagus, clearly by peristalsis. The rate was about the same as for solid food. In both these experiments, the addition of water would sometimes cause irregularities in the descent. Microscopic sections from four different parts of the œsophagus of the goose showed no histological difference.

In the experiments on the cat, the animal was placed on its back and

[1] MELTZER: Journal of experimental medicine. 1897, ii, p. 457.

left side on a holder. The extremities were secured by straps. The head was held between two upright rods connected above by a thong; this allowed free movement of the head without resistance to the passage of food. Shreds of meat dipped in bismuth were ordinarily masticated and swallowed without difficulty. For soft solids bread and milk were used, so fluid as to be easily drawn up into a pipette. The insolubility of the bismuth salt rendered the study of liquids more difficult. Strong solutions of potassic iodide and other salts and suspension of bismuth in acacia and molasses were tried; but a simple mixture of milk and bismuth, shaken in a test tube and immediately drawn up into a pipette, was found most practicable.

Inasmuch as the movement of these different foods varied in different parts of the œsophagus, it will be convenient to divide the latter into three sections. The first or cervical portion extends from the pharynx to the thorax, the second or thoracic from here to the lower half of the heart, and the third comprises the rest of the canal. The relative length of these three parts is about in the ratio of 9:8:6.

The beginning of deglutition was noted by one observer by a finger on the larynx; the same observer called out when the bolus arrived at the thorax, heart, and stomach respectively, while the other observer noted the time. The movement of solids will first be considered. The descent the entire way was by peristalsis, but the rapidity varied. The duration of the movement in the cervical portion was two and a half seconds, and in the thoracic region a little less than two seconds. At the lower end of the heart there was sometimes a slight pause. In the lower section, from the heart to the stomach, the movement was decidedly different. The rate was always very slow. The distance was less than one-third of the entire canal, yet the time consumed in this part ranged from six to seven seconds, or three-fifths of the entire time of descent. The character of the movement here was also peculiar. Whereas in the upper sections the passage was uniform and regular, with a slight acceleration in the thoracic region, here it was apparently irregular, for the bolus descended about one centimetre with each inspiratory movement of the diaphragm, and remained stationary or descended very slightly during expiration. Thus a series of hitches seemed to carry the bolus to the cardia. A probable explanation of this peculiar movement is that the stomach and lower œsophagus were pulled down with each descent of the diaphragm. This would make the movement appear irregular although it was really a slow peristalsis.

It may be well to remark here that this movement was invariably observed in the cat with every kind of food.

Semi-solids, namely, a mush of bread and milk, descended in the same way as solids; but the rate was slightly faster in the upper œsophagus, for the bolus took about a second less to reach the cardiac level. From here the rate was the same as with solids.

For liquids one and a half to two seconds sufficed for the descent to the midheart region. Here there often occurred a long pause — from a few seconds to a minute or more. Then the œsophagus apparently contracted above the liquid, which slowly passed on to the stomach as already described. Sometimes it seemed as if a swallowing movement, evidenced by a rise of the larynx, started the peristaltic wave. Again, several swallows would succeed one another before the liquid passed on. A few times the bismuth and milk seemed strung out along the œsophagus; some more liquid descending would gather this up, and the whole mass assuming an ovoid form would move into the stomach.

Thus in the cat the total time for deglutition varies from nine to twelve seconds. The lowest section presents no change ascribable to a difference in consistency, while in the upper sections the rate does slightly increase with the more liquid character of the food.

In experiments on the dog, bismuth enclosed in capsules or wrapped in shreds of meat was fed as the solid. The general phenomena were as follows. With the rise of the larynx there was a quick propulsive movement of the bolus, which descended rapidly for a few centimetres, sometimes as far as the clavicle. From this point the rapidity was diminished; yet no pause was observed; the bolus simply moved more slowly. This rate was then continued to the stomach without a slackening of speed in the diaphragmatic region, as was observed in the cat. Semi-solids moved in the same way as solids. The total time of descent from larynx to stomach was from four to five seconds.

Liquids gave even a more decided squirt in the beginning of the movement. To render the œsophagus as lax and free as possible, the head of the dog was released from the upright rods and held by the hands after the food was placed in the mouth. Sometimes the liquid descended rather rapidly as far as the heart, at other times no further than the clavicle; then without a pause it passed on slowly and regularly, reaching the stomach in about the same time as solids and semi-solids.

Thus in the dog and cat but little variation was seen in the swallowing of liquids and solids. The liquids pass somewhat faster in the upper œsophagus. But in some animals the difference of rate with foods of varying consistency is much more marked. In the horse, for instance, mere observation shows a decided variation in the rate of movement in the œsophagus. Liquids shoot along the gullet, while solids move clearly by peristalsis. To determine the rate of solids one hand was placed on the larynx of a horse to note the beginning of swallowing and the other hand near the shoulders, where the bolus could be easily felt in its passage. The time consumed by the bolus in passing over a certain distance was measured by a stop watch. The rate obtained for solids, such as hay or grain, was from thirty-five to forty centimetres a second.

For semi-solids, a mixture of bran and water was made, thin enough to run easily between the fingers. Each bolus was watched by a separate observer with a separate watch. The average rate obtained was the same as for solids.

Liquids in the horse pass with a rapidity too great to be affected by peristalsis. Another force must be sought. Among the various muscles supposed to be effectual in moving food into the pharynx, the mylohyoids were shown by Meltzer[1] to be essential. The styloglossi were cut by him without much interference with deglutition, but section of the mylohyoid nerves rendered the act impossible. The activity of these muscles in the horse during swallowing is easily perceived by the hand. Their energetic contraction is a sufficient explanation of the rapid passage of water through the œsophagus. The motion here is more than five times as rapid as that of solids and semi-solids.

Meltzer's experiment to measure the rate of liquids in man by passing a stomach tube containing litmus paper was repeated by us with some modifications. Congo red paper was used, since it is more sensitive than litmus; it also furnishes a means of differentiating between mineral and organic acids, as the discoloration produced on Congo red by mineral acids is removed by ether. It was thus possible to distinguish between the discoloration produced by gastric regurgitation and that produced by the swallowed liquid. For the swallowed liquid one-half per cent lactic acid was found most satisfactory, as the color produced by it on Congo red test paper is almost instantly discharged in ether. By this method the paper

[1] Kronecker and Meltzer: Archiv fur Physiologie, 1880, p. 209.

was found discolored within half a second after the rise of the larynx, certainly too short a period for a peristaltic wave to carry the liquid to the neighborhood of the cardia.

The X-ray method lends itself less successfully to the study of deglutition in man than in the other animals we have studied. The thickness of the thorax, the distance of the œsophagus from the surface, and the relation to dense tissues, render the observation of a swallowed mass difficult, especially when the mass is in rather rapid motion. The few observations which we have to report were made on a seven year old girl placed in the sitting posture. Gelatine capsules containing bismuth were used for solids, and were traced to a point below the heart. The motion was very regular, and apparently due to peristalsis, for the bolus descended without a hitch or irregularity of any kind. Sometimes the capsule became fixed in the upper œsophagus at about the level of the second rib. Repeated swallows of water would fail to dislodge it. An interesting point was noted here. With each attempt at swallowing, the capsule would rise slightly as if the œsophagus was pulled up with the rise of the larynx; then the capsule would descend to its former position.

Semi-solids — a mush of bread and milk — could be seen about as far as solids, *i. e.* to just below the heart. The motion of the mushy bolus was the same as with solids, except that the rapidity was perhaps slightly greater.

It should be noted here that with the human subject, as well as with the horse, our results for semi-solids differ from those derived by Meltzer's method; for according to his statements semi-solids, like liquids, are squirted down the œsophagus and are not propelled by peristalsis, as has been the case in our observations.

Liquids — bismuth and water — were seen only in the neck and upper thorax. Here there was a decided squirt. With the rise of the larynx the liquid was seen to pass rapidly through the pharynx and well down into the thoracic œsophagus before it was lost to observation. The rate, however, by estimation was less than that of liquids in the horse.

There remains to be considered Meltzer's latest investigation,[1] in which he endeavored to ascertain whether liquids remain above the cardia till the arrival of the peristalsis, or ooze down before. An experimental answer was secured by Meltzer by the following

[1] MELTZER: Journal of experimental medicine, 1897, ii, p. 453.

method. The abdominal and gastric walls of an anæsthetized dog were incised and a tube (vaginal speculum) introduced. Through this the entrance of food into the stomach could be observed directly. In repeated experiments no liquid was seen to pass through the cardia before the arrival of the peristaltic wave. An incision through the diaphragm near its anterior origin showed that the swallowed liquid was not squirted as far as a point an inch above the diaphragm. To observe the œsophagus nearer its beginning, the upper three ribs were resected on the left side. Thus the swallowed liquid was seen to shoot along the œsophagus before any peristalsis reached this point. The resection of the fifth rib exposed the œsophagus half way between the bifurcation of the trachea and the diaphragm. Here a bulging was sometimes observed immediately after the beginning of the act, and the swallowed mass remained there until a peristaltic wave carried it down. If the mass swallowed was small, or was projected with moderate force, it might not even reach as far as the bifurcation. From these experiments Meltzer concluded that in animals as in man, liquid food is not carried down the œsophagus by peristalsis, but is thrown rapidly into a deep part of the canal. The depth reached depends on the quantity swallowed, the force used, and the tonicity of the lower part of the œsophagus.

The difference between these methods of Meltzer and those employed in our experiments has already been mentioned; and merely his results, which were obtained with liquids alone, need be considered here. According to our observations on the dog, there was no distinct pause at any part of the canal. The movement simply became slower, and continued at this rate until the stomach was reached. Neither was the rate through the diaphragmatic part of the œsophagus slower than through the thoracic. The quick propulsive movement noticed in the dog was observed with solids and semi-solids as well as with liquids, but the liquids descended further down the canal before the movement changed to the slower peristalsis. While this difference was evident to the eye, the total time consumed by liquids in passing from pharynx to stomach was not enough shorter than the time for solids and semi-solids to be determined by our measurements.

SUMMARY.

The phenomena of œsophageal deglutition as determined by our experiments may then be described as follows: —

There is a difference in swallowing according to the animal and the food which is used.

In fowls the rate is slow and the movement always peristaltic, without regard to consistency. A squirt-movement with liquids is manifestly impossible, as the parts forming the mouth are too hard and rigid. With this diminution of propulsive power in the mouth there is observed a greater reliance on the force of gravity. The head is raised each time after the mouth is filled, and the fluid by its own weight trickles into the œsophagus, through which it is carried by peristalsis.

In the cat the movement is always peristaltic and slightly faster than in fowls. A bolus takes from nine to twelve seconds in reaching the stomach. Liquids move somewhat more rapidly than semi-solids in the upper œsophagus. In the lower or diaphragmatic part the rate is very much slower than above, and is the same for liquids as for solids.

In the dog the total time for the descent of a bolus is from four to five seconds. The food is always propelled rapidly in the upper œsophagus and moves more slowly below. This rapid movement is frequently continued further with liquid food. No distinct pause was observed when the movement of the bolus changed from the rapid to the slower rate.

In man and the horse liquids are propelled deep into the œsophagus at a rate of several feet a second by the rapid contraction of the mylohyoid muscles. Solids and semi-solids are slowly carried through the entire œsophagus by peristalsis alone.

A CONTRIBUTION TO THE CHEMISTRY OF CYTOLOGICAL STAINING.

By ALBERT MATHEWS.

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IT has long been known to histologists that different elements of the cells and tissues show affinity for different stains. Many nuclei, some mucins, and hyaline cartilage stain powerfully in methyl green, Bismarck brown, thionin, and other basic stains, while other nuclei and most cytoplasmic elements show a decided preference for eosin, acid fuchsin, acid green, and other acid stains. The nature of the chemical reactions upon which this elective staining power rests has never received adequate attention. It is several years since Ehrlich[1] classified all stains as "acid," "basic," and "neutral," yet it is still uncertain upon just what properties the affinity of chromatin for basic dyes and cytoplasm for acid dyes really depends. It is still not uncommon to find in cytological works methyl green and other basic stains regarded as microscopical reagents for the detection of chromatin, and some cytoplasmic bodies because of their affinity for basic dyes have been looked upon as chromatin or derivatives of chromatin.

The first observations on the possible chemical basis of the staining reactions of chromatin were made by Miescher,[2] who found that nucleinic acid, a component of chromatin, formed green insoluble salts with methyl green. Lilienfeld[3] called attention to the same fact and referred the affinity of the chromatin for basic stains to the formation of these salts. Lilienfeld[4] also advanced our knowledge by showing that albumin stained pre-eminently in the acid stains and nucleinic acid only in the basic. In studying the artificial nucleins he found that they possessed a varying affinity for acid or basic stains according as the nucleinic acid was more or less completely saturated with albumin. He also observed that egg albumin precipitated by

[1] EHRLICH: Archiv für Physiologie, 1879, p. 571.

[2] MIESCHER: Verhandl. d. naturf. Gesellsch. in Basel, 1874, vi, p. 138.

[3] LILIENFELD: Archiv für Physiologie, 1893, p. 391.

[4] LILIENFELD: Archiv für Physiologie, 1893, p. 554.

alcohol stained neither in acid nor in basic stains. Lilienfeld believed that the affinity of the cytoplasm for acid stains was due to its containing much albumin, but he made no suggestion as to the nature of the combination of the stain with the albumin molecules. He fell into error in supposing that albumin stained only in the acid stains. It will be shown farther on that under suitable conditions the albumin molecule may be made to combine also with the basic stains. In practical experience histologists have observed that sections stained in acidified solutions of the Biondi-Ehrlich mixture take chiefly the acid stain, while in alkaline solutions they take the basic. No explanation of the cause of this phenomenon has been offered, so far as I am aware.

The present paper presents the results of experiments which give some indication I believe of the probable nature of these affinities, and which also show how far cytological stains may be used as accurate micro-chemical reagents. It must be understood at the outset that the results here recorded of experiments on egg albumin, albumoses, and peptones can be directly applied only to such sections of tissues as have been killed and fixed in alcohol or acid media free from metallic salts such as mercuric and platinic chlorides; and further that the conclusions do not apply to those staining processes which probably involve the precipitation of the coloring matter in the tissue, such as the iron-hæmatoxylin method.

I. Experiments on Albumoses.

A. **The acid stains.** — Physiological chemists are aware that albumin and the albumoses react like weak bases, and that they will combine with free acids. If acetic, hydrochloric, or sulphuric acid is added to a solution of albumoses it may be shown by appropriate methods that the acids have chemically combined with the albumoses, although no precipitate is thrown down. Many other free acids enter into similar combinations with the albumins and albumoses, but form insoluble compounds, thus precipitating the albumoses from solution. If a solution of picric acid is brought into a solution of albumose a precipitate consisting of the picric acid combination of the albumose is thrown down. The same kind of reaction ensues with meta-phosphoric, molybdic, wolframic, phosphor-wolframic, tannic, stearic, or chromic acid. Only the free acids will combine with the albumoses. If a neutral solution of the salts of the above-mentioned acids is added to a neutral solution of the albumoses no

reaction occurs. A few drops of acetic or hydrochloric acid are necessary to call forth the reaction. On the addition of acetic acid to a mixture of albumose and sodium picrate, a precipitate consisting of the picric acid combination of albumose appears at once. Probably the reason is that the acetic acid sets free the picric acid, which at once combines with the albumose molecule.

It occurred to me that the so-called "acid" stains, which are generally the sodium salts of sulfonic acids, probably combine with the albumin molecule in the same manner as the above mentioned acids. Experiment fully bore out this hypothesis, for I found that the acid stains possess the same albumin-precipitating powers as sodium picrate or wolframate. The addition of acid fuchsin, acid green, nigrosin, anilin blue black, erythrosin, congo red, carminate of soda, methyl blue, indigo carmine, or other acid stain to a solution of albumoses or albumin gives no reaction. If, however, a few drops of dilute acetic acid be added to the mixture of albumose and acid stain, the colored combination of the stain with the albumose is at once precipitated. One can indeed use this test for detecting the presence of albumin or albumoses in solution or for distinguishing between acid and basic stains, as the basic stains do not give this reaction. This reaction of the acid stains indicates beyond doubt that these stains when in acidulated solutions will enter into chemical combination with the albumose or albumin molecule like any other acid. Inasmuch as it is probable that the free acids enter one or more of the basic NH_2 groups of the albumin molecule, the acid stains also probably enter this group.

B. **The basic stains.** — The basic stains react with the albumoses very differently from the acid. The former stains are generally the chlorides or hydrochlorates of colored organic bases. They react as might be expected like other organic bases. It is well known that in alkaline solution many metals may be made to form combinations with the albumin molecule. If for instance lead acetate is brought into a neutral solution of albumoses or albumins no reaction occurs. If now the solution be made slightly alkaline with sodium carbonate a precipitate is formed consisting of a lead compound of albumin. It is probable that the lead enters the albumin molecule in a different place from the acids already mentioned, and that it enters the hydroxyl of the phenol group, since gelatine and protamin, which lack this group, are not precipitated by basic lead acetate. Many organic bases react like lead.

Protamin, histon, and quinine — strong organic bases — precipitate albumin and the albumoses in alkaline solution. The basic aniline colors react similarly. They may in this manner be made to form colored combinations with albumin and the albumoses.

If basic fuchsin, methyl green, thionin, safranin, or other basic stains (with the possible exception of vesuvin), are brought into a neutral or slightly acid solution of the albumoses, no reaction takes place. If on the other hand they be brought into solutions of the albumoses made slightly alkaline with sodium carbonate a flocculent, colored precipitate consisting of the albumose in combination with the dye is thrown down. This reaction may be used to distinguish the basic from the acid dyes. Vesuvin is precipitated by sodium carbonate alone, but if albumoses are present it is possible, though I have not specially examined the matter, that the precipitate is a combination of vesuvin with the albumose.

These experiments prove that many of the basic dyes enter into chemical combination with the albumose molecule when in alkaline solutions, forming insoluble colored compounds. They show also that in acid or neutral solution this reaction does not occur.

To sum up: (1) The acid stains will combine with albumoses only in acid solutions. (2) Under such circumstances they form combinations similar to picric or other acid combinations with albumoses, and probably enter one or more NH_2 groups in the albumose molecule. (3) The basic stains will combine with the albumoses only in alkaline solution, when they form insoluble colored compounds. The basic dyes react in this respect like basic lead acetate, protamin, histon, or other organic bases. (4) The basic stains probably enter the hydroxyl of the phenol group of the albumose molecule, since they will not precipitate gelatine.

II. Coagulated Egg Albumin.

A. **The acid stains.** — Coagulated egg albumin reacts toward the acid stains like the albumoses. If egg albumin coagulated by heat or alcohol be brought into neutral or alkaline solutions of the acid dyes the albumin will not stain. It is true that it will imbibe a certain amount of color and will appear stained, but this color is easily and quickly removed by washing in water. If on the other hand pieces of coagulated albumen be brought into solutions of the acid stains which have been slightly acidulated with acetic acid the albumin

stains instantly and intensely. The color cannot be removed even by prolonged washing. A most striking contrast is shown by two pieces of coagulated albumin, one of which has been immersed in a neutral, the other in an acid solution of acid fuchsin. After washing, the former will be found to be colorless, the latter a brilliant red.

B. **The basic stains.** — Towards the basic stains coagulated albumin reacts on the whole like the albumoses. Egg albumin coagulated by heat is normally alkaline. If its alkalinity be neutralized or if it be brought into a slightly acid solution of the basic dyes it will stain but slightly. Its power of staining under such circumstances I believe to be due to some other constituent than the albumin, possibly to the mucoid matter present. If however the coagulated egg albumin without neutralization be brought into neutral or slightly alkaline solutions of the basic dyes methyl green, thionin, safranin, methylen blue, or toluidin blue, it stains with great intensity and instantaneously. This may be most strikingly seen in the case of thionin or safranin. If two pieces of coagulated egg albumin be brought the one into slightly acid and the other into alkaline solutions of thionin, the stain poured off after a few seconds, and the albumin washed in water, the piece that has been in the alkaline solution will be an intense purple, the other barely tinged with color.

These reactions clearly indicate that the staining of coagulated albumin depends on chemical combinations similar in all respects to those which the albumoses enter into with the same stains. In neutral solution, neutral coagulated albumin combines neither with acid nor basic stains; in alkaline solutions, it combines only with the basic; in acid solutions, only with the acid stains.

III. Carminic Acid, Hematine, and the Action of Aluminium.

Paul Mayer[1] has shown that carminate of soda and hæmatine are plasma stains, as are the acid aniline colors, whereas the aluminium salts of these acids are chromatin stains, as are the basic aniline colors. Carminate of soda and hæmatine react towards the albumoses like the acid stains. In neutral or alkaline solutions they do not combine with the albumoses or albumins; in acid solution they precipitate the albumoses at once. Freshly prepared hæmatoxylin will not stain tissues, and corresponding with this I find that fresh solutions

[1] Mayer: Mittheil. a. d. zoolog. Stat. Neapel, 1892, x. p. 170.

of hæmatoxylin will precipitate the albumoses neither in acid, neutral, nor alkaline solutions. As aluminium gives carminic acid and hæmatine the staining properties of the basic aniline colors, it is of interest to see how these salts react towards the albumoses. I found that the aluminium salts of these acids (Mayer's carmalaun and hæmalaun) would not precipitate the albumoses in neutral or acid solutions. Thus they differ completely from the sodium salts. In alkaline solutions of the albumoses the addition of solutions of carmalaun or hæmalaun caused heavy flocculent colored precipitates. It is possible that the precipitate was simply the stain which is insoluble in alkaline solutions, but it is also possible that it was the stain in combination with the albumose. In any case the aluminium salts of carminic acid and hæmatine no longer react toward the albumoses or tissues like acid stains, but like basic stains. This is possibly due to the strong basicity of the aluminium, and its tendency to form double acid salts. It will probably be found, I believe, that the aluminium salts of the acid aniline colors stain like the basic dyes.

IV. The Staining of Sections.

The foregoing experiments suggest that the affinity of sections of tissues for stains depends upon reactions similar to the above. So far as I have experimented, the results have fully confirmed this suggestion, but the formation of salts by acids of the tissues with the basic dyes also comes into play. We will consider this first.

A. **The basic dyes in neutral solution.** — The basic dyes in neutral or acid solution will not combine either with albumin or the albumoses. It is clear from this that the affinity shown by chromatin, cartilage, and mucin for such dyes when in neutral solution must depend on something else than the albumin molecule. The suggestion of Miescher and Lilienfeld that the affinity of chromatin for the basic dyes depends on the nucleinic acid indicates the essential cause of the staining reactions of the elements just mentioned. Besides the albumin molecules they contain, mucin, chromatin, and hyaline cartilage have little else in common than the presence in each of organic acids in salt combinations with strong bases. There can be little doubt that the basic dyes in neutral solution will stain any element of the tissue which contains an organic acid in a salt combination with a strong base.

That methyl green in neutral or acid solutions stains those chromatins in which the nucleinic acid exists in a salt form is shown by

its striking affinity for the chromatin of some spermatozoa, thymus gland cells, leucocytes, cells of the spleen, and the red blood corpuscles of birds, and by its slight affinity for the cells of the vertebrate pancreas. In the thymus gland, leucocytes, and red blood corpuscles, Kossel[1] has shown the chromatin to be composed largely of the histon salt of nucleinic acid. In the spermatozoa of the fish and sea-urchin, Miescher,[2] Kossel,[3] and the author[4] have shown the chromatin to be either a histon or protamin salt. In the pancreas on the other hand nucleinic acid exists in a much firmer combination. Lilienfeld's[5] observations on the artificial nucleins confirm this also. He found that the artificial nucleins stained in methyl green so long as they were not saturated with albumin. So soon as the acid became saturated with albumin, the nuclein showed a preponderating attraction for the acid stains. This is strong evidence that the acid stain enters the albumin molecule, while the basic enters the nucleinic acid molecule in these nucleins.

Cytoplasmic bodies with an affinity for basic dyes also indicate that these dyes will stain elements containing the salts of other organic acids. Hyaline cartilage possessing an affinity for such dyes consists, according to Schmiedeberg,[6] largely of the potassium or other salt of the chondroitin-sulphuric acid. Many mucins have the same power of staining in basic stains. The chemistry of mucins is not well known, but many of them, at any rate, react distinctly acid.[7] Many other acids which are possibly present in the cell form insoluble colored salts with the basic dyes. If a basic dye is added to neutral soap solutions a flocculent, colored precipitate consisting probably of the colored salt of palmitic or stearic acid is thrown down. Neutral solutions of thyminic acid, a derivative of nucleinic acid, or of the pseudo-nucleinic acid derived from the yolk of hen's eggs show similar reactions.

These considerations permit us to formulate the following conclusions as to the staining powers of the basic stains. In slightly

[1] See LILIENFELD: Zeitschr. f. physiol. Chemie, 1894, xviii, p. 473.

[2] MIESCHER: Archiv fur exper. Pathol. und Pharmakol., 1896, xxxvii, p. 100.

[3] KOSSEL: Zeitschr. f. physiol. Chemie, 1896, xxii, p. 176.

[4] MATHEWS: Zeitschr. f. physiol. Chemie, 1897, xxiii, p. 399.

[5] LILIENFELD: Archiv. fur Physiologie, 1893, p. 391.

[6] SCHMIEDEBERG, O.: Archiv fur exper. Pathol. und Pharmakol., 1891, xxviii, p. 355.

[7] HAMMARSTEN: Zeitschr. f. physiol. Chemie, 1888, xii, p. 189; also Lehrbuch der physiologischen Chemie, ii Aufl., 1896, p. 139.

acid or neutral solutions the basic dyes will stain any element of the tissue which contains an organic acid in a salt combination with a strong base. In no sense are these dyes a test for nucleinic acid or chromatin. All conclusions in regard to the origin of cytological elements from chromatin or their similarity to chromatin based on the staining reaction are hence worth very little. In neutral or acid solutions the basic stains may be used, I believe, as micro-chemical tests of some accuracy for the detection of the salts of organic acids.

B. **The basic stains in acid and alkaline solutions.** — It has been shown above that in acid or neutral solutions the basic stains will not unite with albumin, but in alkaline solution will combine with the albumin molecule. To test the staining reactions of tissues in the basic dyes in the light of this fact, pieces of liver, kidney, and voluntary muscle of the frog were placed in neutral and acidulated ninety-five per cent alcohol. The acidulated alcohol contained one per cent of acetic acid. The tissues were imbedded in paraffine and cut as usual. The fixation was excellent. In staining, all dyes were used in weak aqueous solutions, and the sections were well washed in water before and after immersion in the stain. Sections were left in the dyes from a few seconds to three minutes. If brought into strong solutions of the dyes the tissues imbibe a considerable amount of stain, I presume by a physical process, but this may be entirely removed from the cytoplasm by a comparatively short bath in water or alcohol.

The liver, kidney, and muscle fixed in neutral or acid alcohol give purely chromatin stains with neutral solutions of the dyes vesuvin, methyl green, methylen blue, safranin, toluidin blue, thionin, and dahlia.

In his Vade-Mecum, Lee, speaking of methyl green, insists again and again that the stain must be slightly acidulated with acetic acid. With all basic dyes, I have found the result better if a neutral solution is taken, though slight acidification seems to do nothing more than to diminish somewhat the intensity of the stain. In either case a pure chromatin stain is obtained.

When used in alkaline solutions, the basic stains react otherwise. It has been shown that in alkaline solutions the basic dyes combine with albumin. I find that sections of the above mentioned tissue, if immersed for an instant in one-tenth per cent sodium carbonate solution before staining or if stained in solutions of the basic stains made slightly alkaline with sodium carbonate show the cytoplasm deeply

stained, as well as the chromatin. The stain, even in the cytoplasm, is in such firm combination that it is exceedingly difficult, if not impossible, to wash it out. In this manner the cytoplasm of these cells may be stained a bright green with methyl green, brilliant red with safranin, a deep blue with methyl blue or toluidin blue, and purple with thionin. Vesuvin alone seems to be an exception.

These reactions, which are identical with those of the albumoses, show that in alkaline solution many of the basic dyes will combine with the albumin molecule whether in cytoplasm or nucleus. As we have already seen, they probably enter the phenol group of this molecule. The basic dyes in alkaline solution may thus be used for the detection of albumins in the cell, and indeed of albumins possessing a phenol or tyrosin group.

C. **The acid stains.** — The acid stains do not combine with albumin in neutral or alkaline solutions, but only in acid solution. The tissues show the same reaction. Tissues hardened in neutral alcohol will not stain in neutral or alkaline solutions of the acid stains, even such intense stains as acid fuchsin. If brought into concentrated aqueous solutions of these stains, the sections imbibe a certain amount of stain, more or less difficult to remove by washing. If such sections be run rapidly through the alcohols they will appear stained. That the stain in such sections is not in chemical combination is shown by the fact that in dilute solutions of the stains such imbibition is exceedingly slow or wholly lacking, and also by the fact that even after immersion in concentrated staining solutions the stain may be entirely removed by washing some time in water. If, on the other hand, sections of tissues hardened in neutral alcohol are washed before staining with one per cent acetic acid or are brought into acidulated solutions of the acid stains, indigo carmine, carminate of soda, nigrosin, methyl blue, erythrosin, acid green, congo red, orange G., and acid fuchsin, the cytoplasm stains instantly and intensely. The stain cannot be washed out.

The observation that sections of such tissues as liver, kidney, and muscle will not stain in neutral acid fuchsin appears at first glance to be contrary to the common experience that sections will stain in non-acidulated solutions of this color. The contradiction is only apparent. Nearly all fixing reagents are acid, and the free acid undoubtedly combines with the albumin of the protoplasm. Having acid already in combination it is not necessary to acidulate the acid stains, for the sodium of the stain probably unites with the acid derived from

the fixing fluid, and the acid stain replaces this in the albumin molecule. That this is true is shown by the fact that the same tissues hardened in *acid* alcohol stained readily in neutral solutions of the acid stains.

This staining reaction of the tissues with the acid stains, corresponding as it does with the reactions of the albumoses and albumin, enables us to conclude that the acid stains enter into chemical combination with the albumin molecule in protoplasm and probably with an NH_2 group in that molecule. The acid stains may be used in acid solution on tissue hardened in alcohol or acetic-alcohol, as micro-chemical reagents for the detection of albumin in the cell elements, with the proviso that there may be other unknown basic substances in protoplasm forming similar compounds, and that possibly in some cases the albumin may already be in such combination with other substances that it will not unite with the acid stains.

The observations here recorded by no means elucidate all the phenomena of staining, but, I believe, they indicate one method of attacking the problem. It would be interesting to know what influence the introduction of mercury or other metals and of acids into the albumin molecule may exert on its staining properties. Until this is known the results and conclusions of the present paper cannot be applied to tissues fixed in corrosive sublimate, Hermann's fluid, and many other fixing fluids.

NOTES ON CETRARIA ISLANDICA (ICELAND MOSS).

BY ERNEST W. BROWN, PH.B.

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FROM early times lichens have been utilized as articles of diet for man and domestic animals.[1] First among them in importance as a food-stuff is "Iceland moss" (Cetraria islandica), which seems to have recommended itself because of its large content of carbohydrate matter, the so-called lichen-starch. In its natural form this lichen contains bitter constituents, and these must be removed by treatment with water or weak alkalies before the material can be made into bread, as has been the custom in some northern countries. Rabbits almost invariably refuse to eat the lichen unless it has been rendered more palatable as described.

With reference to the real dietetic value of Cetraria islandica, the following analysis of the commercial material will afford some data.[2]

Analysis of Cetraria islandica (dried at 105° C.)

Total nitrogen	0.56 per cent.
Extractive nitrogen	0.14 "
"Protein" nitrogen	0.32 "
Ether extract[3]	1.2 "
Crude fibre	5.3 "
Ash	2.2 "
Material soluble in 85 per cent alcohol	19.1 "
Soluble carbohydrates (as dextrose)	43.3 "

After successive treatment with gastric juice and amylolytically and proteolytically active pancreatic juice at 38° C. only 32 per cent of the material used was dissolved. The residue resisting digestion contained practically all the original nitrogen (0.33 per cent) of the lichen.

It will be observed that the quantity of proteids present must be small at most. The bulk of the material is made up of soluble carbohydrates. The latter were early made the subject of chemical in-

[1] Cf. ALBERT SCHNEIDER: A text-book of general lichenology, 1897.

[2] The methods of analysis employed were essentially the same as described by L. B. MENDEL: This journal, 1898, i, p. 226.

[3] This consisted of free fatty acids (0.4 per cent) and saponifiable fat (0.62 per cent).

vestigation. Without attempting to recite the older and somewhat conflicting observations, we may refer to the more recent results of Hönig and St. Schubert.[1] These investigators conclude that extracts of Cetraria, obtained with hot water, contain two carbohydrates. The chief one of these, lichenin, forms a difficultly soluble jelly in cold water, an opalescent solution in hot water, is not colored blue by iodine, and does not rotate polarized light; on boiling with dilute acids lichenin yields crystallizable dextrose in addition to dextrins. The second carbohydrate, called lichenin-starch, is regarded by these authors as a soluble modification of ordinary starch. It has also been called isolichenin.[2] Munk[3] states that lichenin is most nearly related chemically to starch, and that it probably undergoes the same fermentative changes in the alimentary canal as are produced by boiling with dilute acids. The following experiments by the writer confirm in part and extend previous observations.

Lichenin. — *Preparation.* — The dry assorted Iceland moss was heated in a steam sterilizing apparatus for several hours with a considerable quantity of water, and the extract then filtered on hot water funnels. The cool filtrates deposited a thick jelly which was thrown upon filters and allowed to drain. The gelatinous mass was redissolved in hot water and reprecipitated repeatedly until the cold filtrates as well as the jelly no longer gave any blue coloration with iodine. The gelatinous substance was next treated with warm alcohol until all coloring matter was removed, then extracted with ether and dried. There resulted an almost white, tasteless, odorless powder, soluble in hot water, insoluble in cold water, free from nitrogenous matter, and yielding about one-half per cent of ash.

Hydration by dilute acid. — In each trial a weighed quantity of lichenin was boiled for twelve hours with two per cent hydrochloric acid, and the resultant sugar determined in the neutralized fluid by the Allihn gravimetric method. The specific rotation was likewise ascertained and osazones were prepared.

[1] Hönig und St. Schubert: Sitzungsb. d. k. Akad. d. Wissenschaften zu Wien, 1887, xcvi, 2te Abth., p. 685. The older literature is referred to here. Cf. also Beilstein: Handbuch der organ. Chemie, 3te Auflage, i, p. 1098.

[2] Cf. Beilstein, *loc. cit.*, p. 1099.

[3] Munk, J. und C. A. Ewald: Die Ernährung des gesunden und kranken Menschen, 1895, p. 102; also C. Voit: Die Ernährung. Hermann's Handbuch der Physiologie, 1881, vi, p. 413.

I. 1.0936 grams lichenin (ash-free) yielded on hydration 1.097 grams dextrose. Assuming a hydration equivalent to that of starch, 1.0936 grams lichenin should yield 1.215 grams sugar.

II. (*a*) In a solution of hydration products containing 1.53 per cent sugar (determined as dextrose), in a 200 mm. tube an average of five polariscopic readings gave a rotation of + 1.6°. Then $(\alpha)_D = + 52.2°$.

(*b*) In a solution containing 0.51 per cent sugar in a 220 mm. tube, an average of six readings gave a rotation of + 0.6°. Then $(\alpha)_D = + 53.1°$.

The specific rotation of dextrose, $(\alpha)_D = + 52.5°$.

III. The osazones of the sugar formed were prepared with phenylhydrazin in the usual manner, and recrystallized four times from alcohol. M. p. 199° – 201° C.

The melting point of phenylglucosazone = 204° C.

The experiments thus indicate an almost complete hydration of lichenin, analogous in its results to the conversion of ordinary starch.

Action of enzymes and dilute HCl. — In order to determine the possible fate of ingested lichenin in the alimentary canal, the behavior of the carbohydrate towards the ordinary amylolytic enzymes was reinvestigated. The following typical experiments are selected from the protocols.

I. A one per cent solution of lichenin in boiling water was prepared and placed in a bath at 38° C. Most of the material stays in solution; a portion separates out at this temperature. Saliva was added and the solution was tested for reducing sugars from time to time, with Fehling's solution. No reaction was obtained after *forty-five* minutes. To one portion ordinary starch paste (one per cent) was now added. The solution reached the "achromic point" to iodine solution[1] in *one* minute and sugar was abundantly formed, thus showing that there was nothing present inhibitory to the action of the enzyme. The other portion of the original fluid was unchanged even after several hours.

II. A very active diastase preparation likewise failed to transform the lichenin to reducing sugar during an hour's action at 38-40° C.

III. To a one per cent lichenin paste was added an amylolytically active pancreatic extract (alcoholic). No sugar was formed, while the unimpaired activity of the enzyme was demonstrated as in Experiment I.

IV. The ash from one gram of lichenin was added to a small quantity of starch paste. There was no inhibition of the subsequent action of saliva.

V. A one per cent lichenin paste was treated with saliva for an hour at 38° C. No sugar was formed. The solution was then precipitated with alcohol and the precipitate redissolved in water. The action of saliva was again tried, with the usual negative result. These operations were repeated four times with similar effects.

From experiments like the above it must be concluded that the ordinary amylolytic enzymes have no noticeable action on lichenin. Berg[2] is reported to have obtained similar results with saliva, malt diastase, pancreatic extract, and gastric juice. Since it has been shown that cane-sugar is readily inverted in the stomach by the

[1] Cf. GAMGEE: Physiological chemistry of the animal body, 1893, ii, p. 57.

[2] BERG: Abstract in Jahresbericht der Chemie, 1873, p. 848.

gastric juice[1] and experiments in this laboratory have shown that inulin — likewise resistant to enzymes — is partly transformed to reducing sugar by the action of dilute HCl (0.2–0.4 per cent), the following experiment was tried.

A one per cent lichenin paste was treated with an equal volume of 0.4 per cent HCl and kept at 38° C. for twelve hours. The test for sugar was negative. The mixture was carefully neutralized and treated with amylolytic pancreatic extract. No sugar was formed. Acid of 0.3, 0.4, and 0.5 per cent strength also gave negative results. Glycogen is likewise resistant to the action of these acids at 38° C.

Feeding experiments. — In view of the behavior of lichenin already recorded, it seemed desirable to ascertain whether this carbohydrate would give rise to a formation of glycogen in the liver as has been found by Miura[2] to occur after inulin feeding. Miura's experiments were followed as a type and protocols are given below.

Two rabbits, weighing 2.2 and 2.3 kilos respectively, were starved for six days. The control animal (2.3 kilos) was killed and the glycogen content of the liver found by the Brücke-Külz method to be 0.286 gram (0.7 per cent). The other rabbit (2.2 kilos) received ten grams of lichenin, suspended in warm water, in five portions through the stomach sound at intervals of two hours. Twelve hours after the last portion was fed the animal was killed. The glycogen-content of the liver was found to be 0.086 gram (0.25 per cent). Another rabbit of 2 kilos, likewise starved, was fed about eight grams of lichenin in several doses. The animal was accidentally killed immediately after a portion had been fed. The liver did not contain a weighable amount of glycogen.

The writer has not succeeded in finding rabbits that would eat any considerable quantity of the lichen itself, even after extraction with potassium carbonate to remove the bitter taste. Further experiments with larger quantities of lichenin are desirable.

Isolichenin. This carbohydrate, to which is due the blue iodine-reaction in the filtrates from the lichenin preparation, has received little investigation.[3] It is in some respects closely related to soluble starch. The amount present in the lichen is decidedly less than the amount of lichenin, and a micro-chemical study shows it to be distributed through the cell walls of both the cortical and medullary portions of the plant. Micro-chemical reactions for cellulose give negative results.

Preparation. — The filtrates from the lichenin were concentrated in vacuo at a low temperature (35°–40° C.). If any remaining lichenin settled out on cooling it was filtered off and the solution was treated with several volumes of alcohol. The somewhat gummy precipitate was redissolved in hot water and again cooled. Further traces of lichenin were removed by filtration from the concentrated fluid; the

[1] Ferris and Lusk: This journal, 1898, i, p. 277.

[2] Miura, K: Zeitschr. fur Biologie, 1895, xxxii, p. 255.

[3] Cf. Berg: *loc. cit.*; Hönig und St. Schubert: *loc. cit.*

isolichenin was reprecipitated with alcohol, extracted with alcohol and ether, and reduced to an almost white powder, containing 0.4 per cent ash. This preparation dissolves with difficulty in cold water, readily in hot water, from which it does not separate on cooling. With iodine solution it gives a blue coloration.

Hydration by dilute acid. — The following data were obtained by the methods already indicated for lichenin.

I. 1.021 grams isolichenin (ash-free) yielded on hydration 1.125 grams dextrose. Assuming a hydration equivalent to that of starch, the yield of dextrose should have been 1.134 grams.

II. (*a*) In a solution of hydration products containing 1.23 per cent sugar (determined as dextrose) in a 200 mm. tube, an average of six polariscopic readings gave a rotation of + 1.25°. Then $(\alpha)_D = + 50.8°$.

(*b*) In a solution containing 1.13 per cent sugar in a 200 mm. tube, an average of six polariscopic readings gave a rotation of + 1.17°. Then $(\alpha)_D = + 51.7°$.

The specific rotation of dextrose, $(\alpha)_D = + 52.5°$.

III. The osazones of the sugar formed were prepared and recrystallized four times from alcohol. M. p. 199° C. The crystals resemble those of phenylglucosazone in appearance and solubility.

The hydration products of the isolichenin thus correspond closely in behavior with those obtained from the lichenin of the same plant.

Action of enzymes and dilute HCl. — Hönig and St. Schubert [1] subjected this carbohydrate to the action of malt diastase at 60° C. for several hours. They observed a rapid disappearance of the iodine reaction and formation of dextrin-like substance precipitable by alcohol. From such observations they class isolichenin — their lichen-starch — with soluble starch. The writer has further studied the action of saliva, diastase, and pancreatic extract. Typical experiments are given below.

I. A one per cent isolichenin solution was treated at 38° C. with saliva. The "achromic point" was reached in about one minute, no erythro-dextrin stage being detected. Digestion was continued for an hour. The solution, tested from time to time, gave a slight reduction (with Fehling's solution) which did not increase in amount. Nylander's reagent gave no test for dextrose. The solution was precipitated with alcohol and the filtrate gave no reaction for sugars after removal of the alcohol. The precipitate of dextrin-like substance gave a slight reduction.[2] A flocky blue precipitate was always present in the test. Towards diastase and amylolytic pancreatic extract isolichenin showed similar behavior.

II. Isolichenin was treated with varying strengths of HCl (0.2–0.5 per cent) at 38° C. for twelve hours. No sugar was obtained in any instance.

[1] Hönig und St. Schubert: *loc. cit.*, pp. 694–696.

[2] Musculus and v. Mering (Zeitschr. für physiol. Chemie, 1876, ii, pp. 410–419) obtained from glycogen and starch achroodextrins which likewise slightly reduce Fehling's solution.

The unusual behavior of isolichenin towards amylolytic enzymes — the formation of dextrins without sugars — recalls the formation (from glycogen) of dystropo-dextrin, an achroodextrin resisting the further action of enzymes.[1]

The peculiar carbohydrates of Cetraria islandica are doubtless merely types of those occurring in numerous other varieties of this group of plants.

[1] SEEGEN: Archiv f. d. ges. Physiol., 1879, xix, p. 106; TEBB, M. C.: Journal of physiology, 1898, xxii, p. 428.

VARIATIONS IN THE AMYLOLYTIC POWER AND CHEMICAL COMPOSITION OF HUMAN MIXED SALIVA.[1]

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SINCE saliva is the product of secretory glands having their periods of comparative rest and activity, it follows quite naturally that this secretion might be expected to show variations in amylolytic power at different periods of the day: *i.e.*, that the secretion obtained after a period of glandular activity might possess less starch-digesting power than the secretion coming from glands which have been in a state of rest — due mainly to variations in the proportion of active enzyme present. Further, the well-known sensitiveness of the amylolytic enzyme to changes of reaction suggests also the possibility of fluctuations in amylolytic power dependent primarily upon changes in the proportion of alkaline-reacting salts contained in the secretion. In spite of the large amount of work of a chemico-physiological nature done upon saliva, these questions have received very little attention. During the past year, however, Hofbauer[2] in an interesting communication has presented a series of results, bearing on the daily fluctuations in the amylolytic power of saliva, but his observations were limited solely to determination of the starch-digesting power at different periods of the day without regard to any possible relationship between the amylolytic power and the chemical composition of the secretion. His results, however, show clearly that human mixed saliva does fluctuate in amylolytic power throughout the twenty-four hours, and further that the starch-digesting power of the saliva secreted before breakfast, for example, is greater than that of the secretion collected after breakfast. Our results afford distinct confirmation of the general truth of this observation. Hofbauer states in his paper that the only previous work bearing upon

[1] A summary of some of the results contained in this paper was presented at the Meeting of the American Physiological Society in December, 1897, and published in the Proceedings of the Society, this Journal, 1898, ii, p. iii.

[2] Hofbauer: Archiv f. d. ges. Physiol., 1897, lxv, p. 503.

this subject is that by Chittenden and Ely.[1] The latter work, however, has no bearing whatever upon the question of possible variation in the amylolytic power of the secretion at different periods of the day. Indeed, in the paper in question it is distinctly stated that "the saliva was collected generally an hour or two after breakfast," with the distinct object of avoiding possible variations in composition due to the period of collection. The sole object of that investigation was to ascertain whether there is any connection between possible variations of alkalinity and the amylolytic power of saliva. The results there reported afford no indication whatever of the relative amylolytic action of the secretion for different periods of the day, since the fluids studied were invariably collected at essentially the same hour. It was ascertained, however, that the alkalinity of mixed saliva as measured by titration with a standard acid, using cochineal as an indicator, was fairly constant for a given individual at a given period of the day (9–10 A.M.), while saliva from different individuals may show a constant difference in alkalinity, although in the majority of cases the alkalinity varied only within narrow limits. In amylolytic action, however, there were no corresponding differences; fluctuations were observed, but within too narrow limits to indicate any tangible relation between the two factors.

It has become the custom to assume that the alkalinity of saliva, as indicated by its reaction toward litmus paper, is due more or less to the presence of sodium carbonate. Thus, in the latest text-book of physiology the statement[2] is made that "the alkalinity of saliva depends upon the presence of sodium carbonate. In man and in the dog the percentage of this salt varies from 0.08 to 0.19 per cent." So far as we are aware, however, there is no justification for this statement. In the earlier work from this laboratory[3] it was stated that the average alkalinity for fifty-one samples of human mixed saliva was 0.08 per cent, "expressed in the form of sodium carbonate." Further, in all the tabulated results contained in that paper, the alkalinity, as measured by titration with standard acid in the presence of cochineal as an indicator, was carefully expressed as "equivalent in Na_2CO_3," this being done to avoid any positive statement as to the exact cause of the alkalinity. Further, in the oft-quoted work of Werther[4] the al-

[1] Chittenden and Ely: American chemical journal, 1883, iv, p. 329.

[2] Text-book of physiology, edited by E. A. Schäfer, 1898, vol. i, p. 504.

[3] Chittenden and Ely: American chemical journal, 1883, iv, p. 333.

[4] Werther: Archiv f. d. ges. Physiol., 1886, xxxviii, p. 293.

kalinity of the saliva of the dog was determined by titration with decinormal sulphuric acid with litmus as an indicator: a method which obviously would throw no light upon the cause of the alkalinity. Moreover, in at least some of the tables containing his results the percentage of alkalinity is expressed as "alkalinity calculated as Na_2CO_3."

Examination of a large number of samples of human mixed saliva obtained from different individuals at different periods of the day convinces us that, under normal conditions at least, human saliva never contains the least trace of sodium carbonate. Toward litmus, lacmoid, etc., human saliva constantly reacts alkaline, but with phenolphthalein it invariably shows an acid reaction, and a certain amount of a decinormal alkali solution is required to bring out an alkaline reaction with this indicator. Further, phenolphthalein is an extremely sensitive reagent for sodium carbonate; a solution containing 0.001 per cent of sodium carbonate will give a pink color when brought in contact with a solution of phenolphthalein. With human saliva, however, we have never obtained any color reaction with phenolphthalein whatever; the solution invariably remains colorless, thus proving that the alkalinity indicated by litmus must be due to some acid salt or salts, like the hydrogen alkali phosphates, with possibly some alkali bicarbonate. The submaxillary saliva of the dog, however, obtained by stimulation of the chorda tympani is usually, at least, faintly alkaline to phenolphthalein;[1] consequently this fluid may owe its alkalinity in part to sodium carbonate. These facts, which admit of easy confirmation, are worthy of some consideration, since they have an important bearing upon the normal conditions governing enzyme action.

I. Relative Alkalinity and Acidity of Human Saliva before and after Eating.

In this series of experiments the saliva was collected from one individual, stimulation of the secretion being effected by chewing a small piece of rubber. About 15 c.c. of fluid were collected each time. The portion collected before breakfast was obtained at 7.30 A. M., half an hour before eating, while the portion collected after eating was obtained fifteen minutes after the close of the meal. The alkalinity

[1] Chittenden: Science, n. s., 1897, v, p. 902. Also Chittenden, Mendel, and Jackson: This Journal, 1898, i, p. 174.

was determined by titrating the saliva (5 c.c.) with a decinormal solution of sulphuric acid, using lacmoid as an indicator, while the acidity was determined by the use of a decinormal solution of sodium hydroxide, the indicator being phenolphthaleïn. The alkalinity was calculated in terms of sodium carbonate, and is also expressed as milligrams of H_2SO_4 (absolute) required to neutralize 1 gram of saliva. The degree of acidity is expressed as milligrams of NaOH (absolute) required to neutralize 1 gram of saliva. Following are the results obtained: —

Time.	Alkalinity.		Acidity.
	Expressed as Na_2CO_3. Per cent.	Milligrams H_2SO_4 to neutralize 1 gram saliva.	Milligrams NaOH to neutralize 1 gram saliva.
Before Breakfast	0.163	0.78	0.11
After Breakfast	0.127	0.61	0.04
Before Breakfast	0.193	0.93	0.06
After Breakfast	0.130	0.64	0.08
Before Breakfast	0.142	0.69	0.06
After Breakfast	0.122	0.59	0.06
Before Breakfast	0.132	0.64	0.10
After Breakfast	0.132	0.64	0.08
Before Breakfast	0.173	0.83	0.08
After Breakfast	0.127	0.61	0.04
Before Breakfast	0.148	0.71	0.11
After Breakfast	0.132	0.64	0.08
Before Breakfast	0.168	0.81	....
After Breakfast	0.127	0.61	....
Before Breakfast	0.122	0.59	0.11
After Breakfast	0.122	0.59	0.08
Before Breakfast	0.148	0.71	....
After Breakfast	0.137	0.66	....
Before Dinner	0.132	0.64	0.08
After Dinner	0.142	0.69	0.02
Before Dinner	0.168	0.81	0.08
After Dinner	0.158	0.76	0.08
Before Dinner	0.153	0.73	0.06
After Dinner	0.158	0.76	0.02

A glance at these results shows that the alkalinity of saliva, as indicated by lacmoid, is noticeably greater in most cases in the fluid secreted after a night's rest, before breakfast, than in the secretion obtained after the glandular activity induced by the morning meal. Before and after dinner, however (1 P. M.), this distinction is less conspicuous. It is also interesting to note that the average alkalinity, expressed in terms of sodium carbonate, is somewhat higher with lacmoid as an indicator than with litmus or cochineal; a fact which would be expected in view of the presence of the hydrogen alkali phosphates contained in the fluid. It is likewise to be seen that the average acidity as indicated by phenolphthalein, though less conspicuous, is also inclined to diminish after eating.

II. Relative Alkalinity and Amylolytic Power of Human Saliva before and after Eating.

In this series of experiments the main object was to ascertain whether there are noticeable variations in the amylolytic power of saliva before and after eating and whether such variations, if existent, run parallel with variations in the alkalinity. As in the previous experiments, the saliva was collected by chewing a small piece of rubber.

Amylolytic power was determined as follows; 5 c.c. of the filtered saliva were diluted with distilled water to 50 c.c.; 10 c.c. of the diluted fluid were then added to 1 gram of pure arrowroot starch made into a paste with 90 c.c. of water, and the mixture kept at 38° C. for half an hour. Amylolysis was then stopped by boiling the fluid, after which the solution, when cool, was made up to 150 c.c. with water and the reducing sugar determined by the Allihn Method, using 25 c.c. of the sugar-containing solution. The results are expressed as milligrams of maltose formed from 1 gram of starch by 1 c.c. of saliva. The data obtained are given on the next page.

From these results it would appear that saliva secreted after a period of glandular inactivity, as before breakfast, is ordinarily possessed of greater amylolytic power than the secretion obtained after eating; results which accord closely with Hofbauer's observations. Before and after dinner (1 P. M.), however, the difference in amylolytic power is less pronounced; a fact which might be expected in view of the short period for recuperation between the breakfast and dinner and because of the more or less constant stimulation of the salivary glands during the waking hours. Further, we see in these results a suggestion of some degree of relationship between the percentage of

Collector.	Time.	Alkalinity.		Amylolytic Power.
		Expressed as Na_2CO_3. Per cent.	Milligrams H_2SO_4 to neutralize 1 gram saliva.	Milligrams Maltose formed by 1 cc. saliva.
R.	Before Breakfast	0.173	0.83	523.4
	After Breakfast	0.127	0.61	511.8
R.	Before Breakfast	0.168	0.81	630.6
	After Breakfast	0.127	0.61	583.8
R.	Before Breakfast	0.148	0.71	562.2
	After Breakfast	0.132	0.64	485.4
R.	Before Breakfast	0.122	0.59	620.4
	After Breakfast	0.122	0.59	534.6
R.	Before Breakfast	0.148	0.71	549.0
	After Breakfast	0.137	0.66	510.5
J.	Before Breakfast	....	....	209.4
	After Breakfast	....	....	224.4
M.	Before Breakfast	0.117	0.56	585.0
	After Breakfast	0.102	0.49	468.6
M.	Before Breakfast	....	....	621.0
	After Breakfast	....	....	537.6
R.	Before Dinner	0.153	0.73	549.6
	After Dinner	0.158	0.76	536.4
R.	Before Dinner	0.142	0.69	582.0
	After Dinner	0.142	0.69	564.6
R.	Before Dinner	....	....	570.0
	After Dinner	....	....	562.8
R.	Before Dinner	0.163	0.78	599.8
	After Dinner	0.158	0.76	606.6
R.	Before Dinner	....	....	594.0
	After Dinner	....	....	547.2

alkaline salts contained in the saliva and its amylolytic power. Before breakfast, for example, the content of alkaline salts and the starch-digesting power of the secretion are greater than in the fluid secreted after glandular activity. At first glance, then, it might seem that the variations in amylolytic action noticed above are due to changes in the proportion of alkaline salts. The objection to this view, however, is that it associates the higher degree of amylolytic power with the

higher percentage of alkalinity, whereas numerous trustworthy experiments tend to show that saliva manifests its highest degree of digestive power in a perfectly neutral fluid.[1] Consequently, if the above variations in amylolytic action are primarily due to changes in the proportion of alkaline-reacting salts, then the higher degree of amylolysis should be connected with the lower degree of alkalinity. As the reverse is true, the more plausible and natural explanation of the results is that the higher degree of amylolysis is connected primarily with the presence of larger amounts of the amylolytic enzyme, and as this is presumably connected with the outpouring of a more concentrated secretion a corresponding increase in alkaline-reacting salts might naturally be expected. Further, in harmony with the latter view it is to be noticed that the secretions obtained before and after breakfast fail to show any close parallelism between the variations in amylolytic power and variations in alkalinity. Thus the most marked differences in digestive power are frequently seen with salivas which show only a slight difference in alkalinity, and on the other hand marked differences in alkalinity may be associated with minor differences in amylolytic power.

III. Alkalinity, Amylolytic Power, and Composition of Human Saliva before and after Eating.

In view of the preceding results, the following set of experiments was tried in which, in addition to alkalinity and amylolytic power, the proportion of dry solids and inorganic salts of the saliva was likewise determined. The dry solids were determined by simply drying a weighed amount of the filtered saliva — usually five grams — on a water-bath and heating at 105° C. in an air-bath until of constant weight. The inorganic salts were then determined by careful ignition of the residue. In some of the following experiments relative amylolytic action was determined by Robert's[2] method, the method being based on the different lengths of time which solutions of different amylolytic power require to digest a certain amount of starch paste to the achromic point. The results obtained by this method are expressed in minutes; *i. e.*, the number of minutes which elapse from the time the diluted saliva is added to the starch paste until the appearance of the achromic point.

[1] Langley and Eves: Journal of physiology, 1883, iv, p. 18. Chittenden and Smith: Studies in physiol. chemistry, Yale University, 1885, i, p. 8.

[2] See Gamgee's Physiological chemistry of the animal body, vol. 2, p. 56.

Following are the results obtained: —

Collector.	Time. Breakfast.	Vol. c.c.	Alkalinity. As Na_2CO_3 Per cent.	Alkalinity. $Mg.H_2SO_4$ to neutralize 1 gram saliva.	Amylolytic power. Mg. maltose formed by 1 c.c. saliva.	Total solids. Per cent.	Organic matter. Per cent.	Inorganic salts. Per cent.
R.	Before	30	0.158	0.76	649.2	1.02	0.77	0.24
	After	25	0.122	0.59	601.2	0.51	0.33	0.17
R.	Before	25	0.163	0.78	651.0	0.86	0.58	0.28
	After	40	0.112	0.55	615.6	0.51	0.30	0.21
R.	Before	20	0.122	0.59	467.4	0.44	0.23	0.22
	After	25	0.102	0.49	491.4	0.40	0.19	0.21
D.	Before	20	0.081	0.39	43.0[1]	0.37	0.21	0.16
	After	20	0.096	0.46	50.0	0.39	0.24	0.15
A.	Before	50	0.153	0.73	12.0	0.45	0.30	0.15
	After	40	0.158	0.76	15.0	0.53	0.34	0.19
B.	Before	40	0.137	0.66	13.0	0.32	0.15	0.17
	After	30	0.132	0.64	8.0	0.37	0.21	0.16

[1] In this and the two following experiments amylolytic power was determined by Robert's method.

In these results we have a suggestion of the same general tendency toward decrease of amylolytic power and lowered content of alkaline salts in the saliva secreted after the morning meal, while as accompanying results we see corresponding fluctuations (although not in all cases) in the proportion of total solids, organic matter, and inorganic salts, thus bearing out the view that the variations in amylolytic power are connected mainly with changes in the general concentration of the secretion. At the same time it is to be observed that the above differences in composition and amylolytic power are much more marked with the individual R than with A, B, and D. In fact, with the latter three individuals there is very little difference in composition in the saliva before and after the morning meal, and further in the third experiment with R the amylolytic power *after* the meal is greater than that of the saliva secreted before eating. These results have led to another series of experiments having in view especially the determination of the fluctuations in the character of the saliva throughout the day.

IV. Variations in the Composition and Amylolytic Power of Human Saliva throughout the Day.

In the first series of experiments under this head the saliva studied was collected by chewing a piece of rubber. The mid-day dinner was omitted; breakfast, however, was taken at 7.50 A. M. and supper at 6.40 P. M. Samples of saliva were analyzed every hour or two throughout the day. Following are the results obtained: —

Date.	Time.	Volume saliva. c.c.	Alkalinity calculated as Na_2CO_3. Per cent.	Amylolytic power. Milligrams maltose.	Total solids. Per cent.	Organic matter. Per cent.	Inorganic salts. Per cent.
Jan. 20	A.M. 7.15 to 7.30	21	0.112	574.2	0.59	0.29	0.30
" 20	7.50 to 8.15, Breakfast						
" 20	8.40 to 8.55	25	0.091	469.2	0.41	0.18	0.23
" 20	10.00 to 10.15	29	0.102	544.8	0.44	0.24	0.20
" 20	11.00 to 11.18	23	0.102	517.2	0.40	0.23	0.17
" 20	P.M. 12.00 to 12.13	21	0.132	183.6	0.39	0.16	0.23
" 20	12.45 to 12.55	19	0.112	280.8	0.40	0.20	0.20
" 20	2.00 to 2.15	21	0.112	270.6	0.37	0.16	0.21
" 20	3.00 to 3.12	21	0.132	217.8	0.44	0.26	0.18
" 20	4.00 to 4.13	22	0.132	382.8	0.47	0.27	0.20
" 20	5.00 to 5.14	23	0.153	575.4	0.54	0.29	0.25
" 20	7.00 to 7.25, Supper						
" 20	8.30 to 8.45	29	0.153	513.0	0.49	0.25	0.24
" 20	10.40 to 10.55	28	0.163	459.6	0.55	0.38	0.17

Here, as in the preceding experiments, there is noticeable the same diminution of amylolytic power, alkalinity, and content of solid matter, etc., in the mixed saliva secreted directly after the morning meal. Of special significance, however, is the marked variation in the values throughout the day, thereby suggesting the existence of a normal curve of secretion. Thus, after the morning meal the saliva shows the effect of the stimulation by its lower content of solids, etc. Soon after, however, there is an upward tendency; the curve rises, and amylolytic power is increased as well as the alkalinity, together with the total solids and organic matter. The inorganic salts, on the

other hand, still remain low. Towards noon time, amylolytic power sinks very greatly, and there is a corresponding drop in the proportion of organic solids, although the alkalinity and inorganic salts still remain fairly high. After this, amylolytic power gradually rises, reaching the maximum again at 5 P. M. with a corresponding rise in alkalinity, total solids, etc. Supper at 7 P. M. apparently causes a slight fall in amylolytic power, together with a fall in the solid matter secreted. At 10.40 P. M. amylolytic power shows a still greater fall, although alkalinity and solid matter are increased in amount.

How far are the preceding variations in the secreted saliva due to the combined influence of taking food and the mechanical stimulation incidental to mastication of the rubber, and how far to a natural variation in the composition of the secretion? This question we have endeavored to answer by noting the variations in the saliva on a day when food was abstained from, and by collecting the saliva without movement of the jaws. This was accomplished by simply resting the head on the hands, with the mouth downwards, and allowing the saliva to drip into a beaker without any unnecessary movement.[1] In this way 15–20 c.c. of saliva were collected in half an hour.

Following are the results obtained: —

Date.	Time.	Volume saliva. c.c.	Alkalinity calculated as Na_2CO_3. Per cent.	Amylolytic power. Milligrams maltose.	Total solids. Per cent.	Organic matter. Per cent.	Inorganic salts. Per cent.
	Midnight						
Jan. 26	11.45 to 12.15	15	0.081	490.4	0.38	0.23	0.15
	A.M.						
" 27	6.40 to 7.30	13	0.088	572.4	0.63[1]	0.47	0.16
" 27	9.30 to 10.00	15	0.092	558.6	....	....	....
" 27	11.00 to 11.30	20	0.071	381.0	0.33	0.18	0.15
	P.M.						
" 27	12.25 to 12.50	17	0.102	441.0	0.37	0.19	0.18
" 27	2.15 to 2.45	16	0.092	347.4	0.32	0.20	0.12
" 27	4.00 to 4.30	19	0.091	416.4	0.35	0.19	0.16
" 27	5.15 to 5.50	18	0.102	423.0	0.35	0.19	0.16
" 27	7.00 to 7.25,	Supper					
" 27	8.30 to 9.00	20	0.102	403.2	0.43	0.28	0.15

[1] This result is of somewhat questionable accuracy, having been obtained with a very small amount of saliva.

[1] See HOFBAUER: *loc. cit.*, p. 503.

A study of these results shows clearly that when the stimulating influences of food and mastication are withdrawn, conspicuous alterations in the composition and physiological action of the saliva are still found, as though there might be a normal curve independent of the fluctuations induced by stimuli. Thus at 11.30 A. M. there is seen the same fall in amylolytic power that was so conspicuous in the preceding experiment. Further, the saliva secreted at 2.15 P. M. shows a diminution in amylolytic power, as noticeable as the diminution frequently observed after a hearty meal. It is thus quite evident that in the absence of food and other stimulation hourly changes in the amylolytic power of mixed saliva may occur just as marked as those noticed in the saliva secreted before and after breakfast. Variations in alkalinity, total solids, etc. are not so prominent. It is to be noticed, however, from the last series of experiments, that in the absence of breakfast there is no great variation in the amylolytic power of the saliva secreted between 6.40 and 11.00 A. M.; consequently we may accept the conclusion, justified by the results of most of our experiments, that the taking of food, as at breakfast, tends to lower the starch-digesting power of the saliva secreted some time thereafter. This being so it seems probable that other forms of stimulation may likewise give rise to a change in the composition and physiological action of mixed saliva.

V. Influence of Various Stimuli on the Composition and Amylolytic Power of Human Saliva.

In this series of experiments the attempt was made to ascertain how far the character of the stimulus modifies the properties of mixed saliva. The special stimuli employed were ether, alcohol, whiskey, and gin. The first two were taken into the mouth in the form of vapor, and the saliva allowed to trickle from the mouth without motion of the jaws, the fluid so obtained being compared with saliva resulting from the mechanical stimulation produced by chewing a piece of rubber. With whiskey and gin, the mouth was well rinsed with the fluid and the saliva collected by allowing it to flow from the corner of the mouth. The control experiments with water were made in the same way; *i.e.*, the mouth was rinsed with water and the saliva allowed to trickle forth. Finally, for the sake of comparison and to ascertain how far two samples of saliva obtained at such close intervals, under similar forms of stimulation, differ from

each other, four control experiments were tried with water and rubber alone.

Following are the results obtained: —

Date.	Time.	Stimulus.	Volume saliva c.c.	Alkalinity calculated as Na_2CO_3. Per cent.	Amylolytic power. Milligrams maltose.	Total solids. Per cent.	Organic matter. Per cent.	Inorganic salts Per cent.
	A.M.							
Dec. 3	11.05–11.30	Rubber	40	0.168	582.6	0.63	0.31	0.32
	11.30–11.50	Ether	30	0.204	624.6	0.76	0.54	0.22
" 9	9.50–10.10	Rubber	30	....	562.8	0.54	0.30	0.24
	10.10–10.30	Ether	25	....	498.6	0.54	0.29	0.31
" 13	11.40–12.00	Rubber	40	0.122	472.2	0.41	0.21	0.20
P.M.	12.00–12.35	Alcohol	28	0.132	510.6	0.43	0.19	0.24
	A.M.							
" 14	10.00–10.30	Water	30	0.061	473.4	0.32	0.19	0.13
	10.30–11.00	Whiskey	35	0.102	485.4	0.42	0.29	0.13
" 16	10.15–10.40	Water	23	0.071	483.6	0.34	0.20	0.14
	10.45–11.20	Gin	24	0.102	642.0	0.53	0.36	0.17
" 17	10.20–10.38	Ether	27	0.122	586.2	0.32	0.16	0.16
	10.45–10.55	Rubber	28	0.183	577.2	0.52	0.24	0.28
" 20	11.15–11.48	Water	24	0.071	606.6	0.68	0.55	0.13
P.M.	12.15–12.45	Water	24	0.102	564.0	0.38	0.27	0.11
Jan. 11	3.03– 3.35	Water	26	0.053	436.8	0.30	0.16	0.14
	4.05– 4.40	Water	30	0.081	532.2	0.35	0.21	0.14
	A.M.							
" 13	11.25–11.40	Rubber	30	0.153	571.8	0.49	0.26	0.23
P.M.	12.10–12.26	Rubber	30	0.261	550.8	0.47	0.24	0.23
	A.M.							
" 14	10.38–10.58	Rubber	34	0.132	577.8	0.50	0.27	0.23
	11.30–11.45	Rubber	32	0.142	594.6	0.51	0.26	0.25

A glance through these results shows at once certain marked differences in the character of the saliva obtained under the different conditions specified. Thus, saliva which flows from the mouth after the latter has been rinsed once with water invariably shows a lower degree of alkalinity, and generally contains a smaller percentage of solid matter, than the secretion obtained by the other methods. In amylolytic power, however, there is great variation; some samples showing a relatively strong amylolytic action, while others with essentially the same degree of alkalinity are much weaker in their

starch-digesting power. Simple mastication of rubber has a marked influence in raising the content of alkaline salts in the saliva, as well as the total inorganic constituents, and there is a tendency toward increase in amylolytic power although the latter is not constant.

As to the influence of alcohol, ether, gin, and whiskey, there is, we think, no question that these agents taken into the mouth change the character of the secretion, increasing its alkalinity, amylolytic power, and content of solid matter. This is certainly true if the secretion so obtained is compared with the saliva flowing from the mouth without stimulation of any kind. Saliva, however, secreted under the stimulation produced by chewing rubber, is, as we have seen, comparatively concentrated, and the difference between the secretion resulting from that method and the fluid coming from ether, alcohol, and other like forms of excitation, without mechanical stimulation, is not so decisive in the above experiments as to make the matter quite clear, especially in view of the fact that two portions of saliva obtained one after the other, by the same method of stimulation, are liable to show marked differences in composition and reaction. Particularly noteworthy is the fact that of two portions of saliva collected one after the other by mechanical stimulation (chewing rubber) or by simply allowing the saliva to flow from the mouth after once rinsing the latter with water, the latter portion of saliva is, as a rule, more concentrated and possessed of higher amylolytic power than the portion first secreted. It is thus obvious that great care must be exercised in drawing deductions from the composition and amylolytic action of mixed saliva when the latter is so prone to vary under what seem to be essentially the same forms of stimulation. It is furthermore equally obvious that the possible causes to which the above variations may be attributed are many, since there are involved three distinct sets of glands in addition to the buccal glands of the mouth cavity. Hence, increase or decrease in amylolytic power, as well as in the general concentration of the secretion, may involve simply an alteration in the relative activity of the individual glands and not be connected primarily with any specific stimulation of metabolic or secretory activity.

However this may be, it is quite clear that the natural variations in the character of the mixed saliva, indicated by the results of the last four experiments of the above series, render it necessary to use great

caution in arranging the conditions under which the experiments are tried. We have therefore repeated the above experiments, choosing for the collection of the saliva a time of day when we have found the mixed saliva most constant in composition; viz., between 9.30 and 10.30 A.M. To be sure, there are variations in the composition and starch-digesting power of successive portions of saliva collected by the same method at this period, but they are relatively small; quite small, indeed, as compared with the variations liable to occur at other periods of the day. The truth of this statement is illustrated by the two following experiments, in which the saliva was collected without stimulation, simply allowing it to flow from the mouth.

Date.	Time.	Volume saliva c.c.	Alkalinity as Na_2CO_3 Per cent.	Amylolytic Power. Milligrams maltose.	Total Solids. Per cent.	Organic constituents. Per cent.	Inorganic salts. Per cent.
	A.M.						
Feb. 3	9.32 to 10.06	21.0	0.0816	569.4	0.50	0.31	0.19
" 3	10.15 to 10.42	22.0	0.0918	549.0	0.46	0.29	0.17
	P.M.						
" 3	5.00 to 5.20	19.5	0.0918	573.6	0.49	0.31	0.18
" 3	5.27 to 5.50	17.0	0.1122	613.8	0.68	0.51	0.17

Thus, the two portions collected between 9.32 and 10.42 A.M. are essentially alike, while the two fractions secreted between 5.00 and 5.50 P.M., all without stimulation, are more dissimilar. Adopting the morning hour as the better time for collection, experiments were tried with alcohol, ether, chloroform, whiskey, and gin, comparing in each case the saliva obtained under their influence with the secretion coming without stimulation of any kind. The exact method pursued in the case of the control, *i. e.*, with water, was to rinse the mouth once with distilled water after which the saliva was simply allowed to drop from the mouth into a beaker. With ether and chloroform the mouth was filled once with the vapor and the saliva then allowed to flow spontaneously into a receptacle without any motion of the jaws. With the alcohol, gin, and whiskey 10 c.c. of the fluid were taken into the mouth, held a moment, and then ejected, after which the saliva was collected as in the other cases. Lastly, an experiment was tried (Feb. 15) by chewing rubber as a stimulant, and comparing the

saliva so obtained with a control secreted without stimulation. Following are the results obtained:

Date.	Time.	Stimulus.	Vol. saliva c.c.	Alkalinity as Na_2CO_3 Per cent.	Amylolytic Power. Milligrams maltose.	Total solids. Per cent.	Organic constituents. Per cent.	Inorganic salts Per cent.
	A.M.							
Feb. 7	10.05–10.32	Water	18.0	0.0714	480.6	0.42	0.22	0.20
	10.37–10.56	40% Alcohol	18.0	0.1122	514.2	0.43	0.26	0.17
" 8	9.37–10.05	Water	18.0	0.0612	566.4	0.42	0.25	0.17
	10.11–10.32	Ether	18.0	0.1122	558.6	0.54	0.29	0.25
" 10	9.53–10.18	Water	17.5	0.0816	604.2	0.51	0.33	0.18
	10.27–10.47	Chloroform	17.0	0.0714	644.4	0.69	0.48	0.21
" 11	9.40–10.07	Water	17.0	0.0714	493.3	0.39	0.25	0.14
	10.14–10.36	Whiskey	17.0	0.1020	547.8	0.50	0.31	0.19
" 15	9.52–10.16	Water	16.5	0.0816	541.2	0.38	0.21	0.17
	10.21–10.27	Rubber	17.0	0.1530	577.2	0.58	0.26	0.32
" 18	9.33–10.03	Water	17.0	0.0714	584.4	0.49	0.33	0.16
	10.10–10.34	Gin	19.0	0.1020	610.2	0.57	0.39	0.18
" 23	9.26– 9.51	Water	17.0	0.0714	429.6	0.30	0.18	0.12
	10.01–10.24	Water	17.5	0.0714	423.0	0.31	0.18	0.13

From these results it would seem quite clear that the several agents employed, with the exception of chloroform, give rise to a marked increase in the content of alkaline-reacting salts in mixed saliva. Mechanical stimulation, as by chewing rubber, however, is even more effective than the chemical stimuli employed, although it must not be overlooked that in the above experiments the action of alcohol, ether, whiskey, etc., is necessarily of short duration. Further, there is evidence in most of the results of an increase in amylolytic power, as well as in the content of solid matter under the influence of the stimuli. It is thus safe to assert that alcohol and alcoholic fluids not only stimulate the flow of saliva, but that they also tend to increase the concentration and amylolytic power of human mixed saliva, — results which are in close accord with the action of these fluids upon the secretion of the sub-maxillary saliva of the dog.[1] Further, simple mechanical stimulation, as mastication, may also

[1] See CHITTENDEN, MENDEL, and JACKSON: This journal, 1898, i. p. 167.

increase the amylolytic power of mixed saliva. Lastly, it should be mentioned that the saliva resulting from the above forms of stimulation, excepting mechanical stimulation, is much more viscid than the fluid secreted spontaneously, evidently from a higher content of mucin.

Summary.

Human mixed saliva contains normally no sodium carbonate whatever; the alkalinity indicated by litmus, lacmoid, etc., is due to hydrogen alkali phosphates, with possibly some alkali bicarbonate. Mixed saliva invariably reacts acid to phenolphthalein.

The alkalinity of mixed saliva, as indicated by lacmoid, is greater before breakfast than after the morning meal; a conclusion which stands in direct opposition to the statement frequently made that "the alkalinity (of mixed saliva) is least when fasting, as in the morning before breakfast, and reaches its maximum with the height of secretion during or immediately after eating." [1]

Saliva secreted after a period of glandular inactivity, as before breakfast, manifests greater amylolytic power than the secretion obtained after eating, as observed by Hofbauer. Corresponding with this increase in amylolytic power occurs an increase in the proportion of alkaline-reacting salts, but the increased amylolysis is due primarily to an increase in the amount of active enzyme contained in the saliva.

Mixed saliva, whether collected by mechanical stimulation or collected without effort, shows a natural tendency to vary both in composition and in amylolytic power throughout the twenty-four hours, and apparently independent of the taking of food. Between 7.00 and 11.00 A.M., however, in the absence of food the secretion is remarkably constant.

Mechanical stimulation, as chewing a tasteless substance, and alcohol, ether, gin, whiskey, etc., taken into the mouth, all lead to the outpouring of a secretion richer in alkaline-reacting salts and in amylolytic power than the secretion coming without stimulation.

Mixed saliva resulting from stimulation with ether, alcohol, etc., contains a much larger proportion of mucin than the secretion coming without stimulation, being noticeably thick and viscid. This quality is not apparent in the saliva resulting from mechanical stimulation.

[1] Text-book of physiology, edited by E. A. Schäfer, 1898, i, p. 344.

THE VENOMOTOR NERVES OF THE HIND LIMB.

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ALTHOUGH several investigations of the venomotor nerves of other regions, particularly the portal vein, have been published, the literature of the venomotor nerves of the hind limb is limited to the single paper of Thompson.[1] On stimulating the sciatic nerve or the spinal cord of four dogs, Thompson observed that the superficial veins of the hind limb were constricted. The constriction did not extend throughout the vein exposed, but was limited to short sections, between which the diameter remained unchanged. The same result was obtained in four of the five rabbits used.

In my experiments rabbits and cats were employed. The cat is much more satisfactory than the rabbit. The sciatic nerve was severed under ether and the peripheral end stimulated with a weak interrupted induction current while the superficial veins on the outside of the hind limbs were examined. Contractions of the skeletal muscles were prevented by curare. At first the aorta was ligated before stimulation — to exclude the possibility of a decrease in the diameter of the observed vein in consequence of constriction of the arteries of the limb.[2] The veins were kept covered by the skin when not actually under examination. Closing the aorta did not cause any marked decrease in the diameter of the vein, but merely a flattening and general flabbiness throughout its extent. The stimulation on the other hand caused a marked constriction, which was quite irregularly localized. Usually the constricted segments were short, but occasionally a piece ten or twenty millimetres in length would contract uniformly. After a brief exposure to the air the contractions were more variable than at first, parts that had formerly contracted now often failing.

More uniform results were gained when the vein was kept from drying and cooling by irrigation with warm normal saline solution. A flap of skin was raised to form a small reservoir for the saline

[1] THOMPSON: Archiv für Physiologie, 1893, p. 102.

[2] The closing of the aorta was omitted in the later experiments on cats, as it was found to make no essential difference in the result.

solution, in which the vein lay exposed. The part contracted by stimulation was now much longer. Thus in the rabbit the vein occasionally contracted uniformly over a length of seventy millimetres, and contractions of thirty to forty millimetres were the rule. In the cat, the length usually contracting was even greater. But even with the warm saline solution the phenomena were not constant, some parts tiring rapidly and failing to constrict after several stimulations, while others continued with hardly diminished vigor. The position of the latter was usually the same in different individuals. A part of the vein about twenty millimetres in length — just before the vein leaves the surface and passes between the underlying muscles to enter the pelvic cavity — never contracted in any of the rabbits. This part is probably supplied with constrictor fibres through some nerve other than the sciatic, or else the constrictor fibres leave the sciatic on the central side of the point stimulated.

The character of the contraction admits no doubt that it is caused by the vasomotor nervous mechanism. Usually the change in size is considerable, and there is no difficulty in determining whether the vein is constricted or not. Simple inspection of the vein, however, cannot determine with certainty the smaller changes of calibre or the exact time of their beginning. At first an interrupted current that is just distinct on the tongue will usually decrease the diameter of the vein one-third, and sometimes will obliterate the lumen so that no blood can be seen; but the venomotor apparatus is soon tired and then a stronger stimulus is necessary to produce a decided contraction. The latent period is quite long, varying from about ten to twenty or even to thirty seconds. Stronger and more frequent induction shocks decrease the latent period and increase the constriction.

Having determined the presence of venomotor fibres in the sciatic nerve, the next step was to trace them from the spinal cord. For this purpose only cats were used, as they endure the operation much better than rabbits. The animals were anæsthetized with ether during the preparation of the nerves and the vein. The stimulation, which was limited to the peripheral segment of the nerves, was done under curare. The characteristic changes in the vein occurred whether the animal was completely or incompletely under the influence of the drug, but in order to make sure that no activity of the voluntary muscles was responsible for the constriction no results were considered as final unless the curarization was complete.

The part of the hind extremities the veins of which have been particularly examined in determining the course of the venomotor fibres is the lateral surface of the crus, and the pes. All the veins in the former and most of those in the latter region have been seen to contract at one time or another. Since the diagonal vein in the lower part of the crus was the most reliable, the majority of the observations were made in its immediate neighborhood so as to have smaller and fewer cuts in the skin. The veins of the thigh and the medial surface of the crus are apparently less sensitive, for I have as yet not seen them contract; but the number of observations on these veins was small.

To determine the origin of the venomotor fibres from the spinal cord the roots were cut and the peripheral segments stimulated within the vertebral canal. In order to facilitate the operation both the anterior and the posterior roots were tied together outside the dura mater. The cord was removed in the region stimulated so that the possibility of leakage of the current to the cord was excluded. Negative results were never accepted as evidence of the absence of venomotor fibres in the nerve stimulated unless the stimulation of some other spinal nerve, or of the sciatic, gave constriction of the vein and thus proved that the vasomotor apparatus was in working order.

The venomotor fibres to the hind limb, as may be seen from Table I, may be demonstrated in the I to IV lumbar nerves, but in no case were they found in more than three of these in any one animal, and in about half the cases they were found in only two of the nerves. The greatest constriction in every animal but one followed stimulation of the III lumbar nerve. In this exceptional case, the IV lumbar nerve was the most efficient. As this was the only instance in which the lumbo-sacral plexus was of Langley's posterior type[1] it may be that in this type the IV nerve is commonly the most effective. The nerves that produced the most vigorous contraction also influenced a greater length of the vein. There was no definite localization of the area supplied by one nerve, such as was observed later when the gray rami communicantes were stimulated.

From the spinal cord the venomotor fibres enter the sympathetic system. It is *a priori* probable that their course is through the white rami of the spinal nerves, the stimulation of which produces contraction. The highest part of the sympathetic that has given any contraction of the vein is immediately below the III lumbar ganglion.

[1] LANGLEY: Journal of physiology, 1894, xvii, p. 296.

TABLE I.

VENOMOTOR FIBRES IN THE SPINAL NERVES.

Number of Experiment.	Thoracic.	Lumbar.							Sacral.				Character of Plexus.
	xiii	i	ii	iii	iv	v	vi	vii	i	ii	iii	iv	
IV	..	..	..	..	..	o	..	..	..	..	..	..	....
VI	..	..	..	..	..	o	o	..	..	..	..	..	....
VII	..	..	..	..	..	..	o	o	..	..	..	..	....
VIII	..	..	..	..	..	..	..	..	o	o	..	..	....
IX	..	c^2	c^2	c^1	..	..	..	..	..	..	..	..	....
X	..	..	?	c	?	..	..	..	..	..	..	..	....
XIII	..	..	..	c^2	c^1	o	..	..	..	..	..	..	Post. b
XIV	..	..	..	..	..	..	..	o	..	o	o	o	Post. b
XV	o	o	o	c^1	c^2	o	..	..	..	..	..	..	Ant.
XVI	..	o	c^3	c^1	c^2	o	..	..	..	..	..	..	Ant.
XVIII	..	o	c^2	c^1	o	..	..	..	..	..	..	..	Ant.
XIX	..	o	c^2	c^1	o	..	..	..	..	..	..	..	Median.
XX	..	o	c^2	c^1	c^2	o	..	..	..	..	..	..	Ant.

O denotes that no venomotor fibres run in the nerve designated, c that stimulation of the nerve gives a contraction of the vein. The exponents 1, 2, 3, indicate the strength of the contraction, 1 standing for the strongest. Langley's (*Journal of Physiology*, 1894, xvii, p. 296) classification of the different types of the lumbo-sacral plexus is followed.

The limit below which no venomotor fibres enter the sympathetic cannot be determined with certainty because it is masked by the fibres descending the sympathetic trunk from the upper white rami. Thus the constriction obtained by stimulation near what should be the lower limit cannot be used as evidence, for it may be the result of the stimulation of these descending fibres which have entered the sympathetic higher up.

From the III to the VI lumbar ganglion the venomotor fibres are found in the main trunk of the sympathetic; they have not yet begun to leave the sympathetic by the gray rami. The evidence for this

consists in the fact that the stimulation of any part of the main trunk of the sympathetic between the III and the VI lumbar ganglia was always followed by contraction, when the cat was in good condition, and section of the main trunk below the point of stimulation always prevented subsequent contraction. This evidence is conclusive, but it may be added that stimulation of the inferior mesenteric ganglia or any of the nerves connected with it invariably gave negative results.

Let us now inquire by what rami the venomotor fibres leave the sympathetic. The results of stimulating the gray rami communicantes of the spinal nerves forming the lumbo-sacral plexus are brought together in Table II. The rami were not stimulated directly, but the main trunk of the sympathetic was cut above and below the ganglion the ramus of which it was desired to investigate, and stimulated above the ganglion. In the case of the sacral rami, however, it was found inexpedient to cut the main trunk below the ganglia, so that the contractions recorded stand not only for these rami but also for any lower ones that may contain venomotor fibres. But on account of the general absence of these fibres in the II sacral and their occasional absence in the I sacral ramus there is no likelihood of their occurrence in any nerves below the II sacral. In stimulating the II sacral the general method was also deviated from in another respect. Instead of cutting and stimulating the sympathetic below the I sacral ganglion, which would have been difficult, the nerve was first stimulated above the I sacral ganglion and then its ramus severed, or easier still the whole spinal nerve severed, and stimulation repeated at the same place.

It will be seen from Table II that the venomotor fibres reach the sciatic by the rami to the VI and VII lumbar and the I and II sacral nerves. In the same animal, two, or more frequently three, rami contain these fibres, but in no case have they been found in all four rami. In every case the VII lumbar ramus contained venomotor fibres, while the VI lumbar and I sacral contained them in about eighty-five per cent of the cases. In one instance only was the II sacral found to give a contraction, but here, although the length of vein influenced was but one or two millimetres, the constriction was very distinct.

The most noticeable feature of the contractions obtained by stimulating the gray rami is their local nature. While the constriction upon stimulating the sciatic or sympathetic is several centimetres in

TABLE II.

Number of Experiment.	Rami to Lumbar and Sacral Spinal Nerves.						Character of Plexus.
	iv	v	vi	vii	i	ii	
XXII . .	o	o	..	..	..	..	Med.
XXXII . .	..	..	o	C^a	C^p	..	Post. a
XXXIII . .	..	..	o	C^{ap}	$o^?$	..	Ant.
XXXIV . .	..	o	C^a	C^p	o	..	Ant.
XL . .	..	..	..	..	C	o	Post. a
XLI . .	o	o	..	..	..	..	Med.
XLIII . .	..	..	C^a	C^{ap}	$C^?$	..	Ant.
XLIV . .	..	..	C^a	C^{ap}	C	o	....
XLV . .	..	$o^?$	C^a	C^{ap}	$o^?$	..	Ant.
XLVI . .	..	o	C^a	C^{ap}	C^{ap}	..	Ant. 14 thoracic vert.
XLVII left	..	..	C^a	C^a	o	..	Post. a
XLVII right	..	..	..	..	C^p	..	....
XLVIII left	..	..	$o^?$	C^{ap}	C^{ap}	C^a	Ant.
XLVIII right	..	..	o	C^a	C^{ap}	C^p	....
XLIX . .	..	..	C	C^a	C^p	o	Post. a
L . .	..	..	$C^?$	C	C	o	Post. b

O denotes that the ramus was stimulated and no contraction obtained, although the stimulation of other nerves produced contraction. C means that the contraction was obtained by stimulating the ramus indicated. Exponents a, p, mean that the anterior or posterior veins only contracted; where no exponent is given, the condition of the cat was such that the localization observed was probably not significant.

length, the constriction caused by the stimulation of some of the rami is but a few millimetres long, and the region affected by one ramus is frequently different from that affected by another. The VII lumbar ramus controls a greater portion of the veins examined than any of the others, though occasionally the I sacral may equal or even exceed it in importance. The region controlled by the VI lumbar ramus is almost invariably quite small, and is confined to the anterior part of the leg. The transition from the contracting to the

inert region is often most abrupt, so that there is not the least difficulty in tracing the distribution of the fibres; but it may also be so gradual that it cannot be definitely located. When the contracting regions are well marked off from the inert ones it can be seen that sometimes the regions controlled by the VI and VII rami overlap, and that sometimes one stops almost exactly where the other begins.[1] But probably more frequent than either of these two arrangements is the one in which the VII ramus constricts the whole of the region that is affected, the anterior part of the same region being also controlled by the VI ramus. The relations between the VII lumbar and I sacral rami are not so definite, though occasionally similar phenomena are observed. In fact all the contractions of the posterior veins are usually less definite and clear-cut.

The constancy in the control of the anterior veins by upper rami is somewhat surprising in view of the variability in other respects. The only decided deviation from this control was in the II sacral ramus (Exp. XLVIII, left). Even in this case, however, the other side of the same animal possessed the normal arrangement.

The most variable quantity in the whole process is the size of all the regions that contract no matter what nerves are stimulated. From a good many cats no contraction at all can be obtained, and from this wholly negative result to the condition in which stretches of eight to ten centimetres contract strongly and uniformly there is every gradation in the size of the contracting region. Even in this variability, however, there is the constant feature that whenever there is any contraction at all it is almost sure to occur at about the middle of a superficial vein on the lateral side of the lower end of the crus, extending from the posterior edge of this member diagonally downwards and forwards to the upper extremity of the foot. When a greater part of the vein contracts it is usually this same region which contracts most strongly; and it is also to this place that fibres from both the VI and VII rami are distributed.

Another variable feature, which may depend somewhat upon the one just discussed, is the number and arrangement of the rami that produce a contraction. A rather close direct correlation between these and the anterior or posterior arrangement of the plexus would be expected, but Table II shows that there is no such correlation, so far as the small number of observations will allow us to judge.

[1] In several such cases I have subsequently stimulated the sciatic and found that it caused contraction of both these sharply differentiated regions.

The whole path of the venomotor fibres from their origin in the spinal cord to their termination in the veins of the hind limb is apparently made up of two neurons. The cell body of one of these neurons lies in the spinal gray matter; its axis-cylinder process, as I have shown, passes through the anterior root of one of the I, II, III, or IV lumbar nerves and the corresponding white ramus into the sympathetic chain, down which it runs for a certain distance, as described above. The cell body of the second or peripheral or sympathetic neuron lies in one of the sympathetic ganglia. The position of these ganglia was determined by Langley's nicotine method. After painting the III, IV, and V sympathetic ganglia, the stimulation of the pre-ganglionic fibres still causes constriction of the veins. The peripheral nerve cells are consequently not in these ganglia. Painting the VI and VII ganglia, however, renders the stimulation of pre-ganglionic fibres ineffective — the veins do not constrict. It is in one or both of these ganglia, then, that the peripheral venomotor neurons for the veins examined have their cells of origin, and it is here that the axis-cylinder process of the spinal venomotor neuron ends. This at least is true of all the cases I have examined, but it is possible that in Langley's more posterior types of the plexus some peripheral neuron cells may lie in the I and II sacral ganglia. This is suggested by the course of the post-ganglionic fibres.

The post-ganglionic fibres usually leave the sympathetic by the gray ramus immediately below the ganglion in which their cells of origin are situated. Thus when the sympathetic trunk is cut both above and below either the VI or the VII lumbar ganglion, the stimulation of the pre-ganglionic fibres between the section and the ganglion causes constriction. When, however, the ganglion is painted with nicotine the stimulation is usually, but not always, ineffective. This shows that the cells of the distal venomotor neurons are usually in the ganglion just above the ramus through which the fibres leave the sympathetic, but that occasionally they are located in a ganglion higher up. Since it has already been shown that stimulation of the I sacral gray ramus usually, and of the II exceptionally, causes constriction, and since, as has just been pointed out, the cells of origin of post-ganglionic fibres are usually situated in the ganglion immediately above the gray ramus in which they are contained, it follows that peripheral neuron cells may be situated in the I and II sacral ganglia, although I have not been able to demonstrate them with the nicotine method.

In general the arrangement of the venomotor nerves here described corresponds to that of the arterial vasomotor and sweat fibres of the hind limb.[1] The location of the ganglion cells and the course of the fibres through the gray rami is the same as that of the arterial vasomotor fibres, except that the stimulation of the II sacral ramus does not usually produce a contraction of the veins; but on the other hand the origin of the venomotor fibres from the spinal cord is more restricted. Bayliss and Bradford,[2] experimenting on the dog, found vasomotor fibres in the XI thoracic to the III lumbar spinal nerves, and Langley, who used cats, found them in the XII thoracic to the IV lumbar, whereas I have found them only in the I to IV lumbar nerves and have obtained the maximum effect from the III lumbar. It is evident, therefore, that the fibres to the superficial veins of the hind limb originate from the lower end of the region supplying all the vasomotor nerves for that member.

In conclusion I wish to express my thanks to Dr. W. T. Porter, at whose instance this work was undertaken and under whose direction it was carried on.

[1] Compare LANGLEY: Journal of physiology, 1891, xii, p. 347; *ibid.*, 1891, xii, p. 375; *ibid.*, 1894, xvii, p. 296.

[2] BAYLISS and BRADFORD: Journal of physiology, 1894, xvi, p. 10.

AN ANALYSIS OF THE ACTION OF THE VAGUS NERVE ON THE HEART.

By L. J. J. MUSKENS.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

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THE well-known experiments of Gaskell and Engelmann have convinced a great number of physiologists that the regular sequence with which the several parts of the heart contract is dependent on the periodic discharge by the sinus of an impulse to contraction, and the transmission of this impulse to the auricle and ventricle.[1] It has been demonstrated that the excitation process, measured by

[1] For the literature concerning the transmission of the cardiac excitation wave the reader is referred to BAYLISS and STARLING: Internat. Monatshefte f. Anat. u. Physiol., 1892, ix, p. 256; also Journal of physiology, 1892, xiii, p. 407. BURDON-SANDERSON and PAGE: Journal of physiology, 1879-80, ii, p. 384. ENGELMANN: Archiv f. d. ges. Physiol., 1875, xi, p. 465. *Ibid.*, 1878, xvii, p. 68; *Ibid.*, 1894, lvi, p. 149. *Ibid.*, 1896, lxii, p. 543. *Ibid.*, 1896, lxv, p. 109. FANO: Archiv ital. de biologie, 1890, xiii, p. 387. GASKELL, Journal of physiology, 1883, iv, p. 43. MCWILLIAM: Journal of physiology, 1886, vi, p. 226. *Ibid.*, 1888, ix, p. 345. MARCHAND: Archiv f. d. ges. Physiol., 1877, xv, p. 511. WALLER: Philosophical transactions, 1889, clxxx, p. 169. WALLER and REID: Philosophical transactions, 1887, clxxiii, p. 215.

the action current, passes as a wave over the auricle and ventricle, and that this excitation wave may be delayed in its course by various artificial hindrances or by the stimulation of the vagus nerve. It has been demonstrated further that the normal interval between the contraction of the sinus and auricle, and the auricle and ventricle, can be increased by the stimulation of the vagi or by the same artificial means that delay the excitation wave in its course within the auricle and ventricle. Upon these facts the assumption is based that the interval between the contraction of the several parts of the heart is due to the delay of the excitation wave by poorly conducting structures uniting these parts. The lengthening of the interval by vagus excitation is explained by a partial blocking of the excitation wave at the sino-auricular or auriculo-ventricular junction. When the block is sufficiently complete, the excitation wave is wholly arrested and the part of the heart between the block and the apex ceases to beat until the block is removed. Irregularities in rhythm and the periodic grouping of beats have also been explained by variations in the conducting power.

Such are the main outlines of the theory of the heart-beat developed by Gaskell, Engelmann, and others.

When the experimental basis of this theory is examined, it is found that while the propositions just enumerated cannot, in my judgment, be denied, they rest as yet on methods and observations that are incomplete in several important respects. Thus the influence of the vagus nerve on the passage of the cardiac excitation wave was studied by Gaskell chiefly in the tortoise. It is desirable that these phenomena be systematically investigated also in the frog, the classical experimental animal. Again, Gaskell did not maintain the normal nutrition of the heart; indeed, the heart was usually wholly removed from the body. To this abnormal nutrition is to be ascribed the fact that my own observations, both in the present work, begun in 1896 in the laboratory of Professor Engelmann in Utrecht, and in my former research on the reflexes obtained by stimulating the frog's ventricle,[1] differ from those of Gaskell in several important respects. For example, I find that the vagus lessens the force of the ventricular beat in the frog, as Gaskell states,[2] only when the frog is bled, or the normal state otherwise impaired. Finally, Gaskell has not apparently systematically recorded in a large number of animals simultaneous

[1] Muskens: Archiv f. d. ges. Physiol., 1897, lxvi, p. 328.

[2] Gaskell: Journal of physiology, 1883, iv, p. 88.

curves of the movements of the sinus, auricle, and ventricle in such a way that the interval between the contractions of the sinus and auricle could be accurately measured. The lack of systematic records of the movements of the sinus obviously precludes the study of details which have an important bearing on the theory of the heart-beat. Engelmann, on the other hand, has not especially studied the action of the vagus in the light of this theory.

For these reasons it has seemed best to submit the action of the vagus on the heart, as well as certain indissolubly connected problems, to a fresh analysis, using for this purpose methods free from the objections which can be urged against much of the work of previous observers. The fruits of this inquiry will assist, I trust, in establishing still more firmly the views set forth above, and will show that the various actions of the vagus nerve upon the heart can all be explained by changes in the conducting power.

I. Methods of Investigation.

1. **The stimulation of the vagus.** — The methods by which the vagus nerve has been stimulated in systematic researches on animals are alike in that they all require the preparation of the nerve by a dissection often long and difficult. Even in the hands of a skilful experimenter the operation can hardly be performed without some loss of blood, and this interference with the circulation, as will be presently demonstrated, affects the action of the vagus nerve upon the frog's heart to an extent hitherto unsuspected.[1] The usual method of stimulation therefore is of little value for the careful analysis of the action of the vagus nerve upon the heart of the animal in which this action can be most satisfactorily studied.

Another though less important defect is that the two vagi often differ considerably in their influence over the heart even in the same animal. This difficulty may be overcome by the excitation of both nerves simultaneously, but the preparation of both nerves involves a greater injury than the preparation of one, and consequently a greater impairment of the nutrition of the heart, upon the full preservation of which depends the normal action of the nerve.

It is plain, then, that for careful work the usual means of vagus stimulation in the frog must be abandoned, and a method found that does not require dissection and that can be employed for both nerves at the same time. Such a method will be now described.

[1] Compare Engelmann: Archiv f. d. ges. Physiol., 1894, lvi, p. 166.

Soon after leaving the cranial cavity the vagus nerve in the frog passes across the levator anguli scapulæ superioris muscle and touches the cartilaginous capsule which contains the middle ear. The nerves may here be approached from the inside of the mouth with electrodes adapted to the local conditions. If such electrodes are pressed gently against the posterior margins of the Eustachian tubes, a weak current will produce in an irritable frog a strongly marked vagus action upon the heart. Not infrequently the stimulation is still more effective when the electrode is placed outside the cartilage ring which supports the Eustachian tube. The electrodes employed (Fig. 1) were made of copper wire insulated by a block of hard rubber and by rubber tubes. The terminals were of lead. The lead terminal pressed with a certain slight force against the mucous membrane, thus making a better contact and assisting to immobilize the frog. For the smaller species of frog (R. temporaria, R. esculenta, R. palustris, etc.) the electrode should be one or two millimetres in diameter; for R. catesbeiana three or four millimetres.

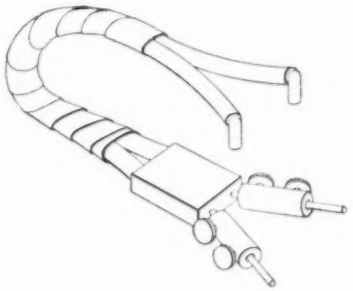

FIGURE 1. Electrodes for the stimulation of both vagi near the middle ear. About one half the actual size.

It may be objected that the part of the medulla oblongata lying between the two electrodes is stimulated by this method, and not the vagus nerve itself. This objection is answered by the following experiment. The electrodes were applied in the manner above described and the minimal stimulation by which arrest of the heart could be produced was determined. The whole central nervous system was then thoroughly destroyed, care being taken not to displace the electrodes. As is always observed after the destruction of the vagus nuclei, the heart was arrested from two to four minutes. After the return of the heart-beat arrest could still be caused by vagus stimulation with the previous strength of current. Occasionally the arrest was more pronounced after the brain and cord were destroyed than before. This experiment was done in curarized and non-curarized Rana catesbeiana and R. palustris.

It may further be objected that the arrest is due to the escape of current to the heart itself. This objection is deprived of its force by

the observation that the effect of vagus excitation was not diminished by increasing the distance between the point of stimulation and the heart. On placing glass tubes 18 to 20 millimetres in diameter in the mouth and œsophagus of several R. catesbeiana the linear distance from the heart-root to the stimulating points exceeded twenty millimetres. The effects of stimulating such a preparation are usually more marked than when the œsophagus is not distended; the nerves are apparently rendered more irritable by being stretched over the glass tube. Additional evidence against the escape of current was secured by laying the nerve of an irritable nerve-muscle preparation upon the heart. Had the very weak stimulating current employed reached the heart, the gastrocnemius muscle must have contracted, yet no contraction occurred. Finally, in two R. catesbeiana very effective vagus excitations were produced when the secondary coil of the inductorium was at 80 and 100 millimetres from the primary. Then both vagi were cut. On repeating the stimulation no vagus effects could be observed even with induction currents of maximal force.

Still another criticism may be made. It may be said that the vagus effects observed were not the result of a direct but of a reflex excitation of the vagi, similar to the reflex excitation obtained by Goltz on striking the intestines. A sufficient answer to this has already been given in the observation that the vagus effect could be secured after the destruction of the cord and brain. It may be added that the latent period in direct stimulation of the nerve is markedly shorter than the reflex time, as determined for example by Engelmann and myself for the reflex from the stomach upon the heart (two to three seconds).

The last objection which occurs to me is that the stimulation by my method affects not merely the vagi but adjacent nerves as well, for example, the sympathetic. This is indeed the case, but it should be remembered that sympathetic fibres join the vagus trunk immediately after it pierces the skull. This objection can therefore be made against any method of vagus stimulation, except the intracranial one, which cannot be employed for the studies we are now making because the formidable operation which it requires disturbs the normal influence of the vagi upon the heart.

2. **The method of recording contractions.** — The movements of the cold-blooded heart may be recorded best by the so-called suspension method of Gaskell[1] modified by Engelmann. Engelmann's modi-

[1] GASKELL: Journal of physiology, 1883, iv, p. 43.

fication has important advantages: it does not interfere with the circulation of the blood; and it has been thoroughly studied with regard to its details. In the suspension method as employed by Engelmann the point of the ventricle *in situ* is pierced by a pin bent at an angle, and the pin is connected to the short arm of a light counterpoised lever. However rough it may seem superficially, the harmlessness and effectiveness of this suspension have been fully proved.[1]

For the present inquiry into the influence of the vagus on the contraction interval it was evidently necessary to record as precisely as possible the time elapsing between the beginning of the auricular and the beginning of the ventricular contraction. The suspension of the ventricle alone has the disadvantage that the deepest point in the curve does not coincide exactly with the beginning of the contraction of the auricle,[2] as can be seen in Engelmann's Fig. 5. In a great number of my experiments, therefore, the ventricle and auricle were suspended separately each to its own lever. By this procedure the auricular lever may be made to write the contractions of the sinus as well as the auricle. In the American bull-frog, which not infrequently attains the length of fifteen inches, the conditions for the record of the contractions of the sinus, auricle, and ventricle are very favorable indeed. After some experience contraction curves ten millimetres and more in height can be obtained from the sinus or one of the large veins. An extremely light lever with a very fine writing point is needed. Very often the movements of that part of the auricle which is closely connected with the sinus superimposes a catacrotic elevation on the sinus curve. This is, however, not an imperfection of method; on the contrary, it permits occasionally a more exact measurement of the interval between the beginning of the contraction of that part of the sinus which is suspended and the beginning of the contraction of the corresponding region of the auricle.

The greatest care should be taken not to injure the delicate muscular walls of the veins. The suspension clamp, made of German silver wire in the shape of a serre-fine, must not be allowed to include

[1] ENGELMANN: Archiv f. d. ges. Physiol., 1892, lii, p. 357.

[2] This imperfection can be remedied to a great extent by making the heart contract more slowly. A test tube filled with ice and placed in the œsophagus will accomplish this by cooling the sinus. An additional advantage is thus secured, for the whole heart is raised upwards and the suspension of its various portions greatly facilitated.

a slip of the pericardial membrane. In all cases the pericardium is to be removed as far as possible before suspending.

3. **The preparation of the experimental animal.** — The immobilization requires great care. Destruction of the brain cannot be thought of; in the first place this operation cannot be done without an abundant extravasation in the brain cavity and the spinal canal, which, as will be seen, disturbs the normal action of the vagus upon the heart; in the second place the rough operation gives rise to countless efferent impulses, which cannot fail to exert an important influence upon so sensitive an organ as the heart. Ordinary doses of curare are also excluded. Curare administered in an amount sufficient to paralyze the voluntary muscles diminishes the reflex irritability and also the effectiveness of the vagus stimulation upon the heart. The most satisfactory manner of preparing the frog is to give an almost homœopathic dose of curare twelve to twenty hours before the experiment. The dose (which is to be determined for every one per cent curare solution used) is about .01 cubic millimetre. As a matter of course the larger animals need more of the poison than do the smaller ones. It can be given by a syringe subcutaneously, or be injected by a pipette into the dorsal lymph-sack. The drug administered in this manner has simply a restraining effect upon the voluntary movements. Within twenty-four hours the animal is generally perfectly restored. If the curare has totally paralyzed the animal in one hour, the chance for good results is already decreased.

The irritability of the experimental animals is a very variable factor. Especially is this true of the frog. In some seasons (spring) it may happen that the excitation of the vagi has a pronounced effect on the heart in almost every frog, while at other times only one animal in ten or twenty may possess sufficient irritability. It was sometimes possible to increase the irritability of the frogs by leaving them over night with exposed intestines in the moist chamber. The best test of a sufficient irritability is the occurrence of spontaneous arrest of the heart. This is often seen in fresh irritable frogs. Thus in slightly curarized animals movements of the limbs having the character of a movement of escape will take place, without any discoverable reason; a latent period follows and then arrest of the heart. Such arrest is often seen to recur in regular intervals of two or three minutes.

This spontaneous — I dare say physiological — arrest gave me an indication of what character and what duration artificially produced

arrest ought to be. The best arrest I hold to be that obtained with currents of minimal intensity and duration.

The vagi, or their terminations in the heart, are very soon fatigued. A rest of at least two minutes between successive stimulations is necessary.

The heart is to be kept moist. Very useful for this is a physiological gelatine solution (5 grams of gelatine dissolved with the aid of heat in 300 grams of normal saline solution).

II. The Effect of Vagus Excitation on the Interval between the Contraction of the Several Parts of the Heart.

1. **The auriculo-ventricular interval.** — The interval between the contractions of the auricle and ventricle is usually prolonged when the vagi are stimulated. Sometimes, however, especially with very weak currents, the duration of the interval is not affected, although the force of the auricular systole is diminished. This is in fact a most

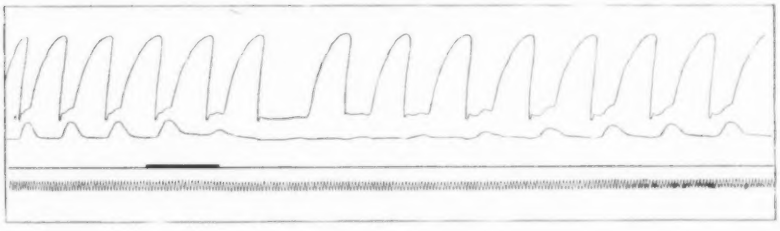

Figure 2. Four-fifths the original size. The uppermost curve records the movements of the ventricle (R. temporaria); the second curve, the movements of the auricle. The elevation in the third curve marks a reflex stimulation from the stomach (tetanization). The fourth curve was written by a tuning fork swinging 10 times a second.

common effect of weak vagus stimulation. In Fig. 2 the intervals are 43, 43, 42, 43, 61, 63, 69, 73, 66, 52, and 46 hundredths second, reading from left to right. As a rule the increase reaches its maximum rapidly and then slowly decreases. A second maximal value is often observed as an after effect.

Figure 3 is an example of the effect of a stronger vagus excitation. The height of contraction is diminished in the first auricular systole after the stimulation, but no measurable increase in the auriculo-ventricular interval is observed until the next cardiac cycle. Then the interval, which had been 39, 40, 40, 40, increases to 63 hundredths

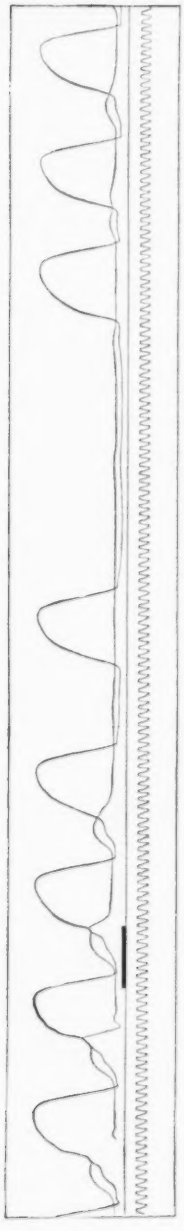

FIGURE 3. Two-thirds the original size. The same as Fig. 2, except that the stimulation was stronger.

second. The ventricle then loses one beat, while only a slight elevation in the auricular curve gives evidence of the systole of the auricle. In the next cycle also the ventricle does not beat, but the vagus influence is already waning, for the force of auricular systole is observed to increase. In the next cycle the ventricle itself contracts again, the auriculo-ventricular interval is still prolonged, being 0.51 sec. Gradually the normal interval and the normal force of auricular systole is restored.

Under still more powerful vagus excitation the auricular systoles become too small to be recorded. The first cycle after the so-called arrest is in such cases often incomplete. The ventricle beats, but the auricular contraction remains invisible, appearing first in the next cycle; or the auricular contractions become visible again, while the ventricular contractions do not yet appear. The effect of powerful vagus excitation on the auriculo-ventricular interval is shown in the accompanying table. This table will serve also to illustrate the method of collecting the data on which the conclusions in this paper rest. A very large number of such measurements were made. Space does not permit their presentation in full; and indeed their full presentation is unnecessary, for they only confirm the conclusion that any one must draw from an inspection of the curves.

The division of the vagus action into the three degrees described above is one of convenience merely. There is no essential difference between them. For the first and second degrees the curves demonstrate clearly that the influence of the vagus on the length of the auriculo-ventricular interval is of the greatest importance. In the third degree the ventricular beat is lost and the auricular

contractions are too small to be recorded; hence we cannot measure the contraction interval; it is, however, certainly prolonged. In the theoretical part of this paper, I shall point out how probable is the view that the apparent arrest of the ventricle is not really an arrest but a failure to be excited to contract, in consequence of the blocking of the excitation wave on its way from the sinus to the ventricle by the action of the vagus.

TABLE I.

Number of Experiment.	Date.	Animal.	Auriculo-ventricular interval in 0.01 sec.[1]											
			Before stimulation.	During and after stimulation.										
1	Jan. 18, 1896	R. temporaria	19	19	13	..	..	..	33	24	22	20	..	..	..
2	Feb. 30, 1896	R. temporaria	41	41	44	..	..	..	?	47	43	42	40	..	..
3	Dec. 2, 1897	R. catesbeiana	40	40	38	..	..	..	..	..	56	54	52	48	44
4	Dec. 7, 1897	R. catesbeiana	50	50	46	50	..	..	..	..	54	52	76	64	56

[1] It should be remarked that the points by which the duration of the contraction interval are measured cannot be located with an accuracy of 0.01 sec. It is not possible to determine so closely as this the exact moment of the beginning of the systolic rise in the curve. The reckoning has been expressed in 0.01 sec. merely to give accuracy to the tenths.

2. **The sino-auricular interval.** — In a second series of experiments the ventricle and auricle were again separately suspended, but the auricular suspension hook was so placed that the contractions of the sinus as well as the auricle appeared in the curve, thus affording the means for an analysis of the interval between the contractions of the sinus and the auricle as well as that between the contractions of the auricle and ventricle. Fig. 4 is an example. In this figure the points at which the contractions of the sinus, auricle, and ventricle begin are indicated by the first, second, and third vertical lines, respectively. The fourth and fifth lines mark the beginning of sinus and ventricle contraction in another period. The sinus continues to beat notwithstanding the vagus excitation, but is much reduced in force, being in the 5th cycle scarcely visible. The force of the auricle diminishes sooner — in the 4th cycle; and in the 5th, 6th, 7th, 8th, and perhaps the 9th, no auricular systole can be seen. In

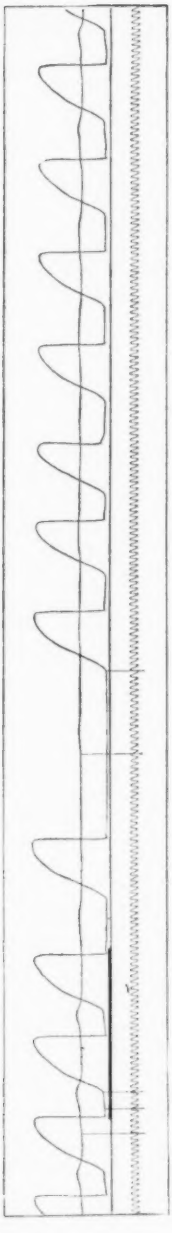

FIGURE 4. Three-fifths the original size. The uppermost curve records the contractions of the ventricle; the next, those of the sinus and auricle; the rise in the third curve marks the reflex stimulation of the vagi (from the stomach); the lowermost curve gives the time in tenths of seconds. The vertical lines indicate the points at which the contractions of the sinus, auricle, and ventricle begin.

the 10th cycle the contraction of the auricle is again visible in the curve and thereafter the force of contraction is slowly regained. The sino-auricular interval cannot be measured here directly, because the auricular contractions are not recorded, but the increase in the interval may be demonstrated by the increase in the interval between the contractions of the sinus and that of the ventricle. If it is urged that this is not a rigid proof, because the lengthening of the sino-ventricular interval may be due simply to the lengthening of the auriculo-ventricular interval, the measurements in Table II, 3 and 4, will be a sufficient answer. These cases are from experiments, in which the auricular contraction, though reduced in height, was still sufficiently visible to make the identification of its beginning-point possible. The interval between sinus systole and auricular systole is considerably increased in case 4. The vagus therefore possesses the power of lengthening the sino-auricular as well as the auriculo-ventricular contraction interval.

The lengthening of these contraction intervals may be regarded as the usual result of vagus excitation. It is, however, not the invariable result. The contraction interval is sometimes decreased. It may even be decreased between sinus and auricle, and at the same time increased between auricle and ventricle, as shown in Fig. 5, or the reversed effect may be observed. The uppermost curve in this figure shows the contractions of the auricle and ventricle, the lowermost curve those of the sinus. The sino-auricular intervals from left to right are 72, 72, 70, 48, 70, and 68 hundredths second, while the auriculo-

ventricular intervals are 42, 44, 42, 50, 46, and 48 hundredths seconds (see Table II, 1 and 2).

3. **The interval between the contractions of the different parts of the sinus.** — Not only may the intervals between the contraction of the main anatomical divisions of the heart, namely, the sinus, auricle, and ventricle, be altered by vagus influence, but the interval between the contractions of different parts of the sinus as well. Evidence will be presented later (Fig. 9) to show that different parts of the sinus may be dissociated by vagus excitation, so that they beat at different times and not practically together as they ordinarily do. The important bearing of this dissociation will be discussed in section V.

TABLE II.

Number of Experiment.	Date.	The sino-auricular and auriculoventricular contraction intervals of successive cycles (in 0.01 seconds).									
		1	2	3	4	5	6	7	8	9	10
1	Nov. 26, 1887	36	18	32	18	33	17	. .	. .	. .	. .
		44	11	46	11	. .	. .	. .	. .	. .	. .
2	Nov. 26, 1887	11	32	18	33	17	. .	. .	. .	. .	. .
		46	11	33	. .	. .	. .	. .	. .	. .	. .
3	Feb. 6, 1886	31	32	50	40	101	56	50	55	49	50
		50	31	32	50	40	101	56	50	55	49
4	Feb. 5, 1886	48	41	50	41	. .	. .	81	43	23	42
		41	50	41	. .	. .	. .	43	23	42	66

[1] Coats: Berichte d. k. Sächs. Gesellsch. d. Wissensch., math.-phys. Cl., 1869, p. 370.

[2] Heidenhain: Archiv f. d. ges. Physiol., 1882, xxvii, p. 395.

[3] Gaskell: Journal of physiology, 1883, iv, p. 88.

[4] Hofmeister: Archiv f. d. ges. Physiol., 1889, xliv, p. 420.

III. The Influence of the Vagus Nerve on the Force of the Heart-Beat.

1. **On the force of the ventricle.** — The vagus is said by Coats,[1] Heidenhain,[2] Gaskell,[3] Hofmeister,[4] and others, to

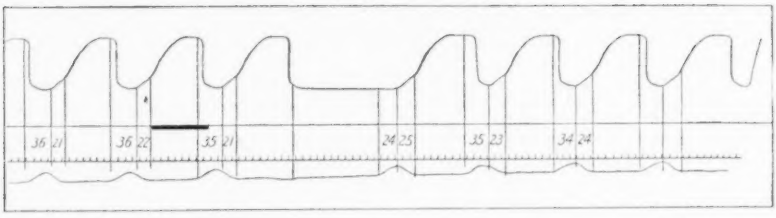

FIGURE 5. Four-sevenths the original size. The uppermost curve records the contractions of the auricle and ventricle; the fall in the next curve marks the duration of vagus excitation; the third curve gives the time in fifths of seconds; and the lowermost curve records the contractions of the sinus (vena cava superior sinistra).

lessen the force of ventricular contraction in the frog. All these investigators employed the excised heart or prepared the vagi without especial regard to the loss of blood. My work with both Dutch and American frogs has shown that the loss even of a little blood alters the action of the vagus on the force of ventricular contraction. Under normal conditions of nutrition I find among thousands of records from four different species of Rana, not one in which vagus excitation diminishes the force in the manner described by these authors.[1] The ventricle, it is true, may be arrested altogether, but this, according to the hypothesis to be presently discussed, is not the result of a reduction in force to the point at which a contraction is no longer possible, but the failure of the excitation wave to reach the contractile substance. Sometimes the height of one or more ventricular systoles after the so-called arrest is found to be irregular — in case the ventricle has missed several beats. The extreme distention of the ventricle with venous blood — mechanically preventing the full effect of the contraction,[2] the slight weakening of the muscle by interference with its nutrition, and the variation in the rate of beat are probable explanations of these irregularities. The simple experiment of bleeding a frog during a continuous series of vagus stimulations has never failed to show that so soon as the normal nutrition is altered

[1] GASKELL, *loc. cit.*, p. 89, found that the vagus does not influence the force of ventricular contraction in the tortoise; MCWILLIAM, Journal of physiology, 1885, vi, p. 223, reached the same result for the eel's ventricle. BAYLISS and STARLING, Journal of physiology, 1892, xiii, p. 411, and NUEL, Archiv f. d. ges. Physiol., 1874, ix, p. 186, deny the power of the vagus over the force of contraction of the ventricle. ROY and ADAMI, Philosophical transactions, 1892, clxxxiii, p. 224, doubt its power.

[2] ENGELMANN, Archiv f. d. ges. Physiol., 1894, lvi, p. 182, points out that the intra-cardiac pressure influences the auriculo-ventricular contraction interval.

even slightly a decrease of the force of ventricular contraction appears. Fig. 6, taken from a frog that had been bled, shows this influence of the vagus stimulation on the force of the ventricle. The last systole before the arrest is already decreased in height.

Sometimes after the arrest the frequency is increased and the force diminished (see Fig. 11, page 507). The latter effect is, I believe, purely secondary. The ventricle beats so quickly that the force of the individual beat is lessened. The increase in frequency is in consequence of interference; the accelerators in the vagus trunk, contrary to the usual rule, probably overcome the inhibitory fibres.

Occasionally though rarely the first contractions after arrest are increased instead of diminished in force. In my experience the increase in force has always been of an irregular type, and associated with a slowing in the rate. The explanation of the increased force is probably the fact established by Gaskell for the tortoise and by McWilliam for the warm-blooded heart, namely, that normally the frequency of beat is too great to permit the ventricle to contract with maximal force. The slow rate under vagus excitation may favor thus the development of the maximal contraction.

2. **On the force of the auricle.** — The action of the vagi upon the force of the auricular contraction has been demonstrated too often to require discussion here. The influence seems to be of the same general character before and after bleeding, although in the latter case it is more marked. As in the ventricle, so

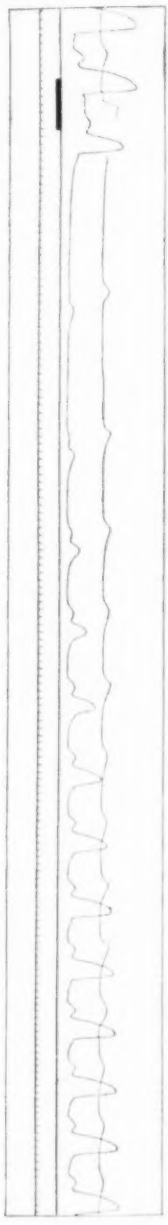

FIGURE 6. Five-ninths the original size. The uppermost curve records the movements of the sinus (vena cava superior dextra); the second curve the movements of the auricle and ventricle; the depression in the third marks the duration of vagus excitation; the lowermost curve marks the time in fifths of seconds.

here the maximum is quickly reached and the previous force regained slowly.

3. **On the force of the sinus.** — The vagi diminish the force of the sinus contraction also; the effect is more quickly produced than in the auricle and ventricle. At times a simple reduction in the height of the curve is observed; at others the systolic rise is no longer single — not one but several elevations are seen at each systole (see Fig. 7). This division of the systolic curve into several parts indi-

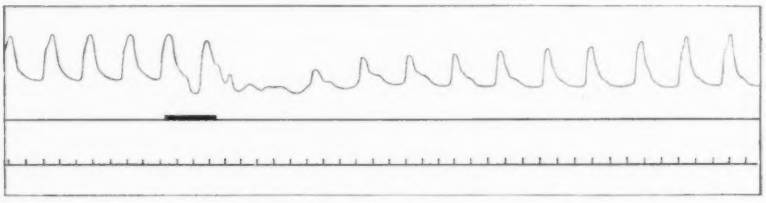

FIGURE 7. Three-fourths the original size. The uppermost curve records the movements of the sinus (vena cava inferior) of Pseudemys elegans; the rise in the second curve marks the duration of vagus excitation; the lowest curve marks the time in fifths of seconds.

cates that the different portions of the sinus no longer contract in their normal sequence, but are dissociated by the vagus. The theoretical importance of this observation will be insisted on hereafter.

IV. THE VAGUS INFLUENCE ON THE FREQUENCY WITH WHICH THE SINUS CONTRACTS.

It will be observed that the title of this section contains no reference to the frequency of the contraction of the auricle and the ventricle. The omission is a logical consequence of the hypothesis which my experiments have forced upon me more and more strongly, namely, that the normal heart-beat is dependent primarily upon impulses discharged by the sinus. This being accepted — and the hypothesis is accepted by a strong school of physiologists — it follows that changes in the frequency of contraction of the auricle and ventricle in consequence of vagus excitation are secondary effects and not the result of the action of the vagi on the production of impulses by the auricle and ventricle. There are two ways in which these secondary effects may be brought about: the vagi may hinder the transmission of the excitation wave and thus delay its arrival in the contractile substance of the auricle or ventricle; or, secondly, the stimulated

vagi may induce intrinsic changes in the auricle and ventricle affecting the character and time-relations of the discharge occasioned by the excitation wave on its arrival in those parts. This being the point of view, we may reserve the consideration of changes in the rate of auricular and ventricular contraction for the discussion of the theoretical bearings of my work. For the present we need only inquire whether any changes in the rate of beat of the sinus are seen when the vagi are stimulated.

The fact is that the rate is often changed. The excitation of the vagus produces sometimes an increase in the frequency of sinus contraction, but usually a decrease.

The occasional increase in the frequency of sinus contraction may be accompanied by an increase in the frequency of the auricle and ventricle. Once I saw after a powerful stimulation of the vagus near its entrance into the sinus a very marked quickening of the heart-beat; this lasted until I stimulated a second time in the same manner and with the same current; then a very marked slowing followed.

Both Dutch and American frogs have furnished me curves in which acceleration and retardation of the whole heart-beat succeed each other suddenly (Fig. 8). This well-known phenomenon is probably

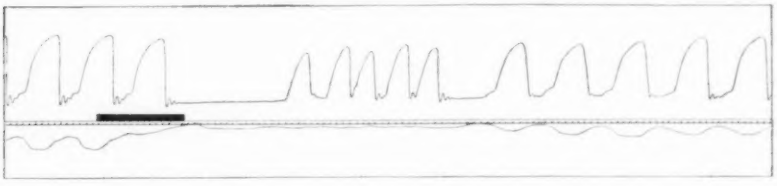

FIGURE 8. One-half the original size. The uppermost curve records the movements of the auricle and ventricle; the rise in the second curve marks the duration of vagus stimulation; the third curve is the time in fifths of seconds; the lowest curve records the movements of the sinus (vena cava superior sinistra).

the result of the interference of retarding and accelerating heart nerves. A satisfactory explanation, in my judgment, can be found in the variation of the conducting power. As soon as the conducting power has increased to a certain point the excitation process, previously blocked, flows over the whole heart and an increase in the number of visible contractions results.

The alternating of arrest with augmented rhythm is apparent rather than real, and is to be explained by changes in the conducting

power in consequence of which the contraction wave is sometimes carried to the ventricle and at other times does not extend beyond its origin in the sinus.

V. The Action of the Vagus explained by its Influence on the Conduction of the Cardiac Excitation Wave.

The basis of the hypothesis about to be discussed is the widely accepted theory that the excitation process which occasions the heart-beat arises automatically in the sinus and from its point or points of origin overflows first the sinus, next the auricle, and lastly the ventricle. In agreement with this theory, the arrest of any part of the heart by the vagus may be explained, first, by the blocking of the excitation wave between its point of origin and the part arrested, so that the latter receives no impulse to contraction; secondly, by a reduction of irritability [1] or other intrinsic change in the arrested part so great that the excitation wave upon its arrival there is not able to occasion any measurable alteration in form. I can offer no rigid demonstration of the truth or error of these hypotheses, but a careful study of the facts leads me to the conclusion that all the various phenomena can be explained by changes in the conduction process so readily and with such close adherence to established facts and scarcely less established points of view as to make this explanation much more probable than the alternative one of intrinsic changes in the several parts of the heart.

1. **The conduction of the excitation process.** — Let us begin our analysis by attempting to explain changes in the duration of the contraction interval by changes in the conduction of the excitation process. In Fig. 9 the uppermost curve records the movements of the auricle and ventricle, the lowermost curve the contractions of the sinus. The time is marked by the chronograph of Jaquet recording fifths of seconds. The current derived from two Daniell cells was conveyed through the primary circuit of an induction apparatus and through an electromagnet which recorded the time of stimulation. The distance between the coils of the induction apparatus was 120 millimetres at the beginning of the stimulation but was slowly reduced to 75 millimetres. On examining the curve it will be seen that the excitation is followed by one normal beat, after which the contractions of the auricle and ventricle disappear from the curve. The elevation observed in the sinus curve is due to the movements of voluntary

[1] Compare McWilliam: Journal of physiology, 1888, ix, pp. 352, *et seq.*

muscles, very often seen in reply to stimulation. Upon close examination, it will be noticed that after the standstill the whole sinus does not contract at the same moment. A small, sharply defined elevation precedes the first large contraction of the sinus by 1.68 secs.; shortly afterward, the contraction wave pursues its regular way over the auricle and ventricle. In the next cardiac cycle, a similar small elevation precedes the main contraction of the sinus by 1.34 secs., — a slight reduction. In the following cycles, the interval between the two parts of the sinus movement falls to 1.14, 0.82, 0.40, 0.28, 0.18, 0.12 secs., and finally the interval disappears and the whole sinus contracts apparently simultaneously.

Evidently the excitation of the vagus in this case dissociated one part of the sinus from another.[1] The normal rapid conduction of the excitation wave in the sinus is here diminished by vagus excitation, just as it has been shown by Engelmann, Gaskell, and others to be diminished in the auricle and ventricle. In consequence of the slower conduction the interval between the part of the sinus first reached by the excitation wave and that reached later is such that the two contractions are no longer fused into an apparently single contraction but are seen separately in the curve. As the vagus influence wanes, the conducting power gradually returns to normal, and the interval between the contraction of the several parts of the sinus becomes less

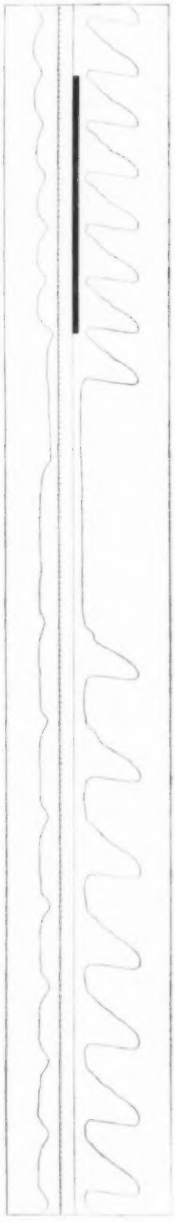

FIGURE 9. Five-sixths the original size. The uppermost curve records the contractions of the auricle and ventricle; the rise in the second curve marks the duration of vagus stimulation; the third gives the time in fifths of seconds; the fourth the contractions of the sinus (vena cava superior sinistra). R. catesbeiana.

[1] McWILLIAM: *ibid.*, 1886, vi, p. 220, observed a similar dissociation in the auricle and sinus of the eel's heart.

and less until fusion again takes place. Sometimes, after the so-called arrest, the sinus is seen to contract in three divisions, and indeed it is entirely probable that the dissociation of the sinus contraction under nerve influence may proceed much further than can be demonstrated by our present methods of research.

Experiments on the sinus in turtles (Pseudemys rugosa and elegans) confirmed the results secured with frogs. In the turtle I succeeded in suspending two different parts of the sinus, *i. e.* two parts of the vena cava inferior, at the same time. Curves were thus obtained which showed that the sinus and the large veins are not one contractile body but a system of contractile units. The contraction wave in most cases was seen to start from the right vena cava. The separation into contractile units may be recognized not only when the heart is exhausted after bleeding (as shown by Engelmann in the frog) and in consequence of vagus stimulation as above described, but also under apparently normal conditions. It seems most probable that the time elapsing between the contractions of the different parts is dependent on the conducting power between these parts.

The reader may be reminded here that antiperistaltic contractions of the sinus and large veins may be observed quite frequently in exposed hearts which are beating normally. I have seen these in the turtle; Engelmann[1] observed antiperistalsis in the frog and Bottazzi[2] in the heart of the chick. The possibility of the occurrence of this phenomenon should be taken into account in the discussion of the cases in which the vagus nerve seems to have a direct influence on the production of automatic stimuli.

If the vagus, as has just been shown, can thus separate the contractions of various portions of the sinus so that a contraction curve that normally appears single becomes divided into several parts, and if, as has been shown in section second, the vagus can also increase the normal interval which separates the contraction of the sinus from that of the auricle, and the contraction of the auricle from that of the ventricle, it is certainly reasonable to suppose that a strong excitation of the nerve may interpose such a resistance to the passage of the excitation wave as to block it entirely. The parts of the heart which cease to receive the excitation, or receive less than the amount sufficient to cause a measurable change in form, will then cease to record

[1] ENGELMANN: Archiv f. d. ges. Physiol., 1895, lxi, p. 275.

[2] BOTTAZZI, P.: Sullo sviluppo embryonale della funzione motoria, Firenze, 1897, p. 78.

their contractions, as, for example, in Fig. 9 the contractions of the auricle and ventricle disappear from the curve for a time, while the sinus goes on beating.

Decreased conducting power within the limits of the sinus will further explain the simple prolongation of one or two cycles often seen as an effect of weak vagus excitation (Fig. 8).

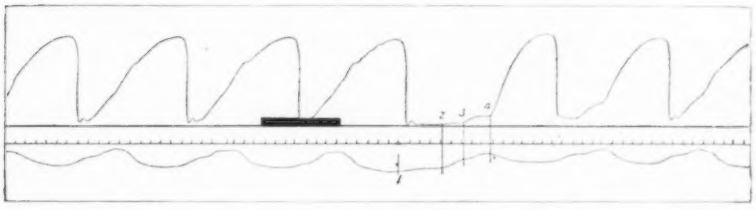

FIGURE 10. The uppermost curve records the contractions of the auricle and ventricle; the rise in the second curve marks the duration of vagus excitation; the third marks the time in fifths of seconds; and the lowermost curve the contractions of the sinus (vena cava superior sinistra). The vertical lines 1, 2, 3, 4 mark the beginning of contraction of the smaller part of the sinus, the greater part of the sinus, the auricle, and the ventricle, respectively.

It also explains numerous instances occurring in R. catesbeiana as well as R. temporaria in which the duration of the cardiac cycle after stimulation is nearly or exactly twice that observed before stimulation (Fig. 5).

2. **The force of contraction.** — The observation that the sinus is really a composite of contractile units, together with the observation that the vagus influences these functional units in different degrees, thus dissociating them from their normal relation, suggests the possibility that the vagus nerve may regulate the force of the cardiac contraction simply by changing the conducting power.

The diminishing force of contraction under vagus inhibition may mean that fewer and fewer fibres take part in the contraction, because the decreasing power of conduction prevents more and more fibres from being reached by the excitation wave.[1] The return of force after vagus inhibition may mean that the improving conducting power enables the excitation to reach a constantly increasing number of fibres. This view is strengthened by the similarity in the action of the vagus on the force of contraction and on the length of the contraction interval; in both the effect rises quickly to a maximum and

[1] Compare ENGELMANN: Archiv f. d. ges. Physiol., 1896, lxii. p. 555.

then slowly disappears. It is further strengthened by the fact that in frogs which have been bled the force of ventricular contraction diminishes nearly *pari passu* with the lessening in conducting power between sinus and auricle, and auricle and ventricle (measured by the increase in the contraction interval).

The diminishing force of contraction may also be explained by the failure of the several parts of the sinus to beat in unison (Fig. 7).

It seems not improbable that the "staircase phenomenon" may be explained by a gradual increase in conductivity enabling each successive excitation wave to reach farther and farther into the ventricle.[1] In this connection it may be remembered that I described in Pflüger's Archiv[2] a peculiar direct effect of tetanizing currents sent through the ventricle, which is to be seen whenever the nutrition of the heart (and also the conducting power) is diminished. I saw the force of the ventricle quickly decrease, and, after the tetanizing, slowly increase again, in other words, a staircase phenomenon. This also may be regarded as due to changes in the conductivity.

3. **The rate of beat.** — The facts already stated warrant the assumption that variations in the duration and the height of the contraction wave of the several parts of the heart, including the total disappearance of a measurable contraction (vagus "arrest"), may be explained by variations in the conduction of the excitation process from one contractile unit to another. We must now inquire whether alterations in the frequency of contraction of the sinus or a part of the sinus can be similarly explained.

In Fig. 11 is presented an example of an apparently complete arrest of the whole heart. The ventricle was suspended in this experiment in such a manner as to permit the contractions of the sinus, auricle, and ventricle to be recorded in one curve. As a result of vagus excitation, all three of these parts seem to be arrested. If, however, the period of supposed total arrest is examined with great care, three regular but very faint elevations will be seen; these are the weakened contractions of the sinus.[3] A single record of these contractions, which are barely measurable, would convince no one. When however it is stated that such records have been repeatedly

[1] Compare ENGELMANN: Archiv f. d. ges. Physiol., 1896, lxii, p. 554.

[2] MUSKENS: *ibid.*, 1896, lxvi, p. 340.

[3] The engraver has very slightly sharpened these elevations in the curve (Fig. 11) in order to show their position clearly.

obtained in my experiments, the significance of these elevations must be conceded. I have observed every degree of lessening in the force of the sinus contraction down to this stage, in which the record trembles on the verge of complete obscurity. The arrest of the heart in these experiments is therefore only apparent. The excitation is still rhythmically discharged, but the resistance to its overflow upon the sinus, auricle, and ventricle is so much increased under the influence of the vagus that the change in form ceases to be measurable in the auricle and ventricle and is barely measurable in the sinus. Evidently should the conduction become still more difficult, even the sinus contraction would cease to be measurable, although contraction might still take place.

The change in rhythm just discussed is only apparent; the true rhythm of the heart, namely, the frequency with which the excitation wave is discharged in the sinus, has been unaltered. But we have to deal also with changes in the true rhythm. It has already been shown that the true rhythm may be increased or diminished by vagus action.

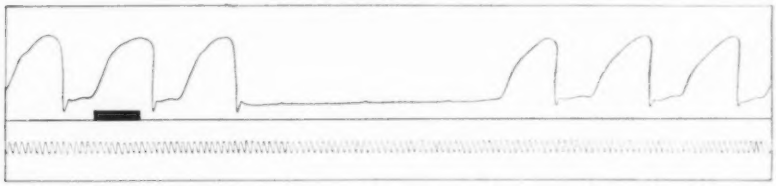

FIGURE 11. Three-fifths the original size. The ventricle was suspended in such a way that the contractions of the sinus, auricle, and ventricle are written in one curve. The black band in the line below marks the duration of the vagus excitation. The time is in tenths of seconds.

An additional example may be cited. In a R. catesbeiana the movements of the auricle and ventricle were recorded in one curve and the movements of the sinus in another. On excitation of the vagus the auricle and ventricle ceased to record, while the sinus beat with increased frequency. These cases can be readily explained by changes in the conducting power.[1] The number of excitation waves discharged from the sinus must depend largely on the resistance to the discharge. If the resistance is great, the threshold value will be high, and the discharge relatively infrequent; if the resistance is slight, the threshold value will be low and the discharge relatively frequent.

[1] Compare ENGELMANN: Archiv f. d. ges. Physiologie, 1896, lxii, p. 552.

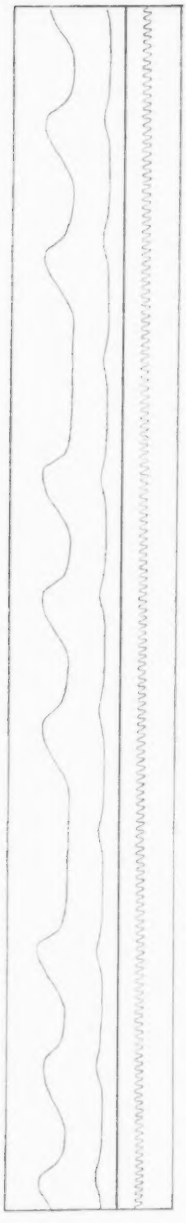

FIGURE 12. Five-sevenths the original size. The upper curve records the contractions of the ventricle; the lower, those of the auricle.

The probability of the vagus increasing or diminishing the resistance to the conduction of the excitation wave has been demonstrated above, and this action of the vagus suggests that the nerve may also regulate the resistance to the discharge of the excitation wave. The latter hypothesis is a simple and, to my mind, easily accepted explanation of the alterations in rhythm often observed when the vagi are stimulated; the threshold value of the excitation discharge is altered under vagus influence.

It should be stated here that an increase in the contraction interval also occurs apparently independently of the vagi in badly nourished hearts. Thus, in the exhausted frog's heart, especially after loss of blood, very interesting changes in the duration of the contraction interval may be observed.

In Fig. 12, the auricle (lower curve) beats with perfect regularity. The contractions of the ventricle are in groups of three. In each group, the auriculo-ventricular interval increases from nearly 0.7 to 1.1 secs. The intervals are 0.94, 1.07, dropped beat; 0.77, 1.01, 1.10, 1.14, dropped beat; 0.80, 1.01, 1.11, dropped beat.

The sino-auricular interval is also sometimes lengthened in badly nourished hearts. Fig. 13 is an example. In this case the sinus beat with absolute regularity (lower curve), but the sino-auricular interval increased until the excitation wave was blocked entirely. The intervals are 1.38, 1.74, dropped beat; 1.16, 1.38, 1.56, dropped beat; 1.16, 1.38, 1.56, dropped beat; 1.16, 1.38, etc.

It seems highly probable that the periodic loss of one beat in the cases illustrated by Figs. 12 and 13 is a consequence of the

continuous increase of the contraction interval. Gaskell [1] and Engelmann [2] have shown that in the heart every contraction reduces the conducting power of the contracting part. In the record before us the auricle contracts with perfect rhythm. At each auricular contraction an excitation wave passes over the ventricle. On the arrival of the first excitation, the ventricle contracts. Its conducting power is lowered by this contraction. Recovery is slow because of imperfect nutrition. The next excitation from the regularly working auricle is delayed by the difficult conduction. Hence the second auriculo-ventricular interval is greater than the first. The excitation from the third auricular beat is delayed like the two preceding ones. The third auriculo-ventricular interval is longer than the second, as the second was longer than the first, for to the delay of the first interval is added the delay of the second. This process goes on. The auriculo-ventricular interval grows longer with each cardiac cycle, as the delay of each is added to the summed delays of its predecessors. Finally, the interval between the beat of the auricle and ventricle exceeds a certain limit, as in the case of vagus excitation (page 493), and a ventricular beat is dropped. The period is complete. The loss of this beat gives time for the conducting power to reach its former level. The next auriculo-ventricular interval is about the length of the first interval of the preceding series, and a new period begins.

This explanation of periodic pulses is of especial interest, for the reason that in badly nourished human hearts, for example in myocarditis and arterio-sclerosis, similar irregularities are not infrequent.[3] It is probable also that many of the

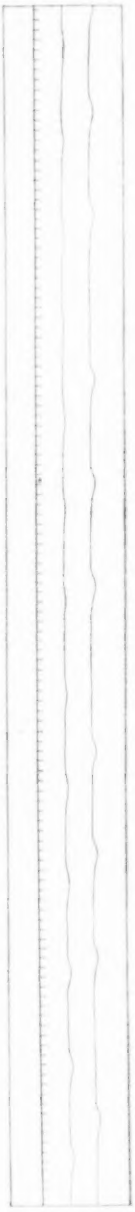

FIGURE 13. Two-thirds the original size. The upper curve records the contractions of the auricle; the lower, those of the sinus.

[1] GASKELL: Journal of physiology, 1883, iv, p. 97.

[2] ENGELMANN: Archiv f. d. ges. Physiol., 1896, lxii, p. 543.

[3] Compare ENGELMANN: *Ibid.*, p. 553.

periodic groups of Luciani (recently studied by Oehrwall[1]) will be thus explained.

In conclusion I desire to express my grateful appreciation of the valuable criticism of Dr. H. P. Bowditch. I am also greatly indebted to Dr. W. T. Porter for assistance in the preparation of my paper for the press.

SUMMARY.

1. The sino-auricular and auriculo-ventricular contraction intervals are usually lengthened by vagus excitation; sometimes, however, they are diminished; the one may be increased, while the other is diminished. The vagus effect quickly reaches a maximum and then slowly decreases.

2. The interval between the contractions of different parts of the sinus is sometimes increased by vagus excitation, so that the different parts are dissociated and beat at measurably different times.

3. The force of contraction of the sinus and auricle is frequently diminished by vagus excitation.

4. The vagus does not diminish the force of ventricular contraction in the frog, except when the normal nutrition of the heart is disturbed by the loss of blood or otherwise.

5. The frequency of sinus contraction is usually diminished by the stimulation of the vagus; at times it is increased.

6. The various actions of the vagus nerve just enumerated, together with Bowditch's staircase, interference, and various forms of irregular pulse, can be readily explained by variations in the transmission of the cardiac excitation.

[1] OEHRWALL: Skandinav. Archiv fur Physiologie, 1898, viii, p. 1.

A NEW METHOD FOR THE STUDY OF THE ISOLATED MAMMALIAN HEART.

By W. T. PORTER.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

THE isolation of the mammalian heart was first accomplished by H. N. Martin,[1] in the Biological Laboratory of the Johns Hopkins University. Normally warmed defibrinated blood entered the right auricle and right ventricle from a reservoir at normal venous pressure. The right ventricle pumped the blood through the lungs, where it was oxygenated, artificial respiration being maintained for that purpose. The pulmonary veins brought the arterialized blood to the left heart, and the left ventricle drove it into a tall tube tied into the aorta, whence the blood was returned to the venous reservoir. The height of the liquid column in this tube determined the pressure against which the ventricle worked, and this "arterial pressure" could be regulated at will.

The advantages of Martin's method are great. The heart is fully isolated from all other organs except the lungs, and works under conditions closely approximating the normal state. Indeed, for many purposes the original plan of Martin is superior to those since advocated. The chief objection to it has been the difficulty of securing blood enough. It is necessary to use dog's blood for the dog's heart, cat's blood for the heart of the cat, etc., and several animals have to be sacrificed in order to secure a sufficient quantity for one perfusion.

In 1890, Martin and Applegarth[2] published an important modification of the procedure just described. In the new method "all the branches of the aorta, except the coronary arteries, are ligated. The venæ cavæ are also ligated. In the aorta itself is placed a cannula, which is connected with a Mariotte's flask, raised a sufficient height above the organ. The defibrinated blood from the flask fills the

[1] MARTIN: Studies from the biological laboratory of the Johns Hopkins University, 1881, ii, p. 119.

[2] MARTIN and APPLEGARTH: Studies from the biological laboratory of the Johns Hopkins University, 1890, iv, p. 275.

connecting tubes, the aorta, and the coronary arteries at a constant pressure, which, of course, is quite independent of the force and the frequency of the heart-beat. The blood taking the coronary circuit, on reaching the right auricle, proceeds to the corresponding ventricle, and from it through the lungs to the left auricle. This blood is, therefore, the only blood entering the cavities of the heart or passing through the lungs unless there be some inefficiency of the aortic semi-lunar valves. That the cavities of the heart are not distended with more blood is not found to influence the normal character of its beat, which continues rhythmically and forcibly for three or four hours."

Arnaud,[1] in 1891, injected defibrinated blood into the aorta of a rabbit the heart of which had ceased to beat, and saw co-ordinated contractions return.

The following year Hédon and Gilis[2] made similar injections in a dog and in an executed man, and secured co-ordinated beats.

Langendorff,[3] in 1895, modified the method of Martin and Applegarth by omitting the lungs, receiving the coronary blood from the right heart into a dish or beaker. The omission of the lungs permits the heart to be removed from the body, an advantage for certain purposes. Langendorff's modification is however open to the objection that the blood cannot be so satisfactorily oxygenated as when it passes through the lungs. Moreover, the removal of the heart prevents stimulation of the extrinsic cardiac nerves.

My own experiments on the isolated heart began nearly a year before the publication of Langendorff's first paper. They were at first directed to the discovery of a method by which the warm-blooded heart could be maintained in rhythmic, forcible contraction by thoroughly oxygenated blood supplied in the normal way, namely, through the right auricle to the right ventricle, thence to the left auricle and left ventricle, and so to the right auricle again. This is not the place to speak of the many devices which were employed one after the other in the attempt to secure a really satisfactory oxygenation of the blood. It is enough to state that none of these devices succeeded in thoroughly oxygenating in a sufficiently short

[1] ARNAUD: Archives de physiologie, 1891, p. 396.

[2] HÉDON and GILIS: C. r. de la soc. de biologie, Paris, 1892, p. 760.

[3] LANGENDORFF: Archiv f. d. ges. Physiol., 1895, lxi, p. 292. Langendorff's modification has recently been employed in altered form by RÜMKE: Geneeskundige Bladen uit Kliniek en Laboratorium, Harlem, 1897, (iv) x, p. 201.

time the quantity of blood required for the successful perfusion of the warm-blooded heart. The attempt was therefore temporarily given over and the method of Martin and Applegarth employed.

My first arrangement of Martin and Applegarth's method agreed with Langendorff's modification in omitting the lungs, but differed from Langendorff's in many other respects. All the branches of the aorta except the coronary arteries were tied. The aorta was kept filled with blood from a reservoir at constant pressure. The semilunar valves being closed by the constant high aortic pressure, the blood was forced through the coronary arteries into the right heart; a very small quantity entered the left heart through the vessels of Thebesius. The force and frequency of the contractions of the left ventricle were recorded by a Hürthle manometer connected with a tube passed into the left ventricle through the left auricular appendix and mitral valve. The blood flowing through the coronary vessels into the right heart escaped through the pulmonary artery on to a registering apparatus, so that the volume of the coronary circulation, excepting the very small quantity reaching the left heart through the vessels of Thebesius, was recorded.

With this method the fact that stimulation of the vagus diminishes the flow through the coronary arteries was discovered,[1] an experiment recently repeated by Maas[2] in Langendorff's laboratory. Curves showing simultaneously the intraventricular pressure and the diminution in the volume of the coronary circulation in vagus excitation, obtained by this method, were shown to the American Physiological Society at its meeting in Boston in December, 1896. One of the curves is to be found in this Journal, 1898, i, p. 160. Another was published in the American Text-book of Physiology, 1896, p. 453, to illustrate the influence of the vagus on the frequency of ventricular contraction.[3] This is the first instance in which a graphic record of the volume of the coronary circulation has been obtained; and the first in which the intraventricular pressure in the isolated heart has been recorded.

With this method also the relation of the volume of the coronary

[1] PORTER: Boston med. and surg. journal, 1896, cxxxiv, p. 39.

[2] MAAS: Archiv f. d. ges. Physiol., 1898, lxxi, p. 399.

[3] In reproducing this curve, the line recording the volume of the coronary circulation was cut out because unnecessary to the illustration of the vagus action on the frequency of the heart: this curve and the one published in the American Journal of Physiology were from the same experiment, March 26, 1896.

circulation to the frequency and force of the ventricular contraction in the isolated heart of the cat was studied. Curves showing the main result of this investigation were published in the American Text-book of Physiology, 1896, p. 476. A detailed account is to be found in the Journal of Experimental Medicine.[1]

In the method last described the animal and the recording apparatus were placed in a huge warm chamber kept at a constant temperature. After a time, I discarded the warm chamber and designed in its stead the apparatus afterwards used by Miss Hyde in her study of the effect of distention of the ventricle on the flow of blood through the walls of the heart. The new method is described in her communication in this Journal.[2]

Meanwhile, I had found that any part of the ventricle of the dog's heart, even the ganglion free apex, will beat for hours if supplied with defibrinated dog's blood through its nutrient artery. By this means the whole ventricle, as well as the apex of the ventricle, was for the first time fully isolated from the rest of the heart and kept in powerful, rhythmic, long-continued contraction.[3] This method has now been constantly used in this laboratory during fourteen months, and can be highly commended. Dogs are usually employed. The animal is anæsthetized with ether, bled from the left carotid artery, the blood defibrinated, and filtered through glass wool. Meanwhile, warm 0.8 per cent sodium chloride solution is allowed to flow into the right jugular vein. After a short interval, the dog is bled again from the carotid artery, and the blood defibrinated as before. The heart is now rapidly removed and placed still beating in a beaker of warm saline solution. Often the beats are so vigorous that the heart with each ventricular systole springs more than an inch from the bottom of the beaker. Thus the organ is self-cleansed from blood. A glass cannula is now tied into the coronary artery supplying the area the contractions of which are to be studied, and the part of the heart wall supplied by the artery is cut out. The cannula bearing the attached ventricular segment is filled with defibrinated blood and joined to a glass tube passing through the rubber stopper of a small glass chamber. A small adjustable clamp sup-

[1] MAGRATH and KENNEDY: Journal of experimental medicine, 1897, ii, p. 13.

[2] Miss I. H. HYDE: This journal, 1898, i, p. 215.

[3] PORTER: Journal of experimental medicine, 1897, ii, p. 391: preliminary account in Journal of the Boston Society of Medical Sciences, 1897, i, issued March 10, 1897.

ports the upper margin of the piece of heart to keep its weight from dragging on the nutrient artery. The chamber is provided with a thermometer. The neck of the chamber passes through a rubber stopper in the floor of a galvanized iron water tank, the sides of which rise above the top of the chamber. A wire attached by a hook to the lower end of the piece of ventricle passes through the neck of the chamber and is fastened to a lever of the second class, the writing-point of which traces the contraction curve, usually magnified seven times, upon a Baltzar drum. Within the water tank also is placed a litre flask filled with defibrinated blood. The contents of this flask are kept at any desired pressure by means of a pressure-bottle. The blood passes from the blood-flask to the heart-chamber and through the cannula in the coronary artery into the ventricular segment, from which it escapes through the severed veins into the lower part of the chamber and runs out into a tall beaker placed to receive it. The blood-flask, the heart-chamber, and the tubes connecting the two are surrounded by a large volume of water at any desired temperature. The water tank is thirty-six centimetres long, twenty-one broad, and twenty-five deep. A window in the front permits a view of the heart as it contracts.

The advantages of this method for studying the fundamental properties of cardiac muscle, the action of animal extracts and drugs upon the heart, and certain other problems, are very great. A large dog's heart affords two and sometimes three separate apex-preparations each with a nutrient artery large enough for a practicable cannula. Several preparations of the basal portion of the ventricle can also be obtained from the same heart. The experiment scarcely ever fails. The perfused piece seldom refuses to beat, and if it does another piece from the same heart can be used. The relatively very large quantity of perfusion fluid allows the circulation to continue for a long time; there is no troublesome turning of stopcocks at frequent intervals. Often the quantity of blood together with the small number of vessels to be supplied makes it unnecessary to perfuse the heart twice with the same blood. The preparation can be made with all care. There is no hurry. Even pieces left for more than an hour will usually beat when perfused. The long survival makes it possible to prolong experiments through most of the day, a fresh piece of ventricle being taken when the one in use wears out. This change of pieces we have found of value in testing the effect of poisons and animal extracts.

Such are the various methods of isolating the mammalian heart At best, they leave much to be desired. They fail to realize the long-deferred hope that the mammalian heart shall be made to beat like the heart of the frog in a Williams apparatus, contracting for hours while fed on a simple perfusion fluid. This result is reached by the following procedure.

It will be remembered that the great obstacle to the perfection of the methods of isolating the mammalian heart has been the difficulty of properly oxygenating the nutrient blood. Oehrwall[1] has shown that even the batrachian heart contracts much more powerfully when surrounded with pure oxygen. Since the publication of Oehrwall's paper many plans for the use of oxygen in the isolation of the warm-blooded heart have been tried by the present writer without avail. In every instance the mechanical difficulties of keeping considerable quantities of blood thoroughly oxygenated have been too great. Not until the discovery that the mammalian heart would beat with a blood-supply much less than is ordinarily thought to be essential,[2] and the further discovery that this relatively small quantity may be effectively supplied to the heart muscle through the veins of Thebesius and the coronary veins[3] did the problem seem once more practicable. On returning to the attack, I determined to feed the heart through these veins in an atmosphere of oxygen, and, if this were not enough, to increase the oxygen pressure, in the hope of thereby facilitating oxidation in the manner taught by Haldane.[4]

The following experiment was accordingly performed.

May 2, 1898. A cat was bled and the blood defibrinated and filtered through glass wool. Cannulas were tied into the right auricular appendix, the pulmonary artery, and the aorta. The cannula in the right auricular appendix led through a Williams valve to a small reservoir of blood. The pulmonary and aortic cannulas were each connected with glass tubes which rose to a short distance above the blood-reservoir and then turned to discharge their contents into the reservoir itself. All the heart vessels except the two arteries mentioned were ligated. The arrangement therefore was closely similar to that of the frog's heart in a Williams apparatus. The heart with its several tubes was now placed in a strong glass cylinder immersed in warm water. The top of the cylinder was provided with a stout brass cap

[1] OEHRWALL: Archiv für Physiologie, 1893, Suppl. Bd., p. 40.
[2] MAGRATH and KENNEDY: Journal of experimental medicine, 1897, ii, p. 13.
[3] PRATT: This journal, 1898, i, p. 86.
[4] HALDANE: Journal of physiology, 1895, xviii, p. 211.

perforated by two tubes. One was a T-tube the side branch of which led to a large metal cylinder containing oxygen under high pressure, while the other branch was provided with a stop-cock opening into the atmospheric air. The second tube led to a pressure gauge. So soon as the oxygen pressure began to rise, the heart, which had ceased to beat, began to contract with great vigor. Surrounding the heart with oxygen even at the pressure of the atmosphere was distinctly helpful, but the contractions became decidedly stronger and more frequent as the oxygen pressure rose. A pressure of about two atmospheres was that ordinarily employed, but as high as four atmospheres was occasionally tried. The blood coursed from the reservoir into the right side of the heart. Each beat of the right ventricle drove blood in a stream through the tube in the pulmonary artery back into the reservoir. The Williams valve prevented regurgitation from the right heart. The heart muscle was nourished through the veins of Thebesius and the coronary veins. A small quantity of blood found its way through foramina Thebesii into the left auricle and ventricle, whence it was pumped by the latter out through the aorta. The vigorous beating of the heart continued from early in the morning until late in the afternoon, when the experiment was broken off. The contractions were vigorous also at room temperature.

Two conclusions may be drawn from this experiment: —

(1) An atmosphere of oxygen is of advantage in maintaining the contractions of the isolated mammalian heart.

(2) A heart fed simply through the veins of Thebesius and the coronary veins will maintain strong, rhythmic contractions for many hours if supplied with oxygen at high tension.

The first thought suggested by these statements is whether the mammalian heart, like the frog's heart, will beat when fed on serum alone, provided that a sufficient supply of oxygen is furnished. The experiment was accordingly repeated on other hearts, but the blood was replaced by serum obtained by centrifugalizing defibrinated blood. As was expected, the absence of corpuscles was readily borne by the heart. Continued rhythmic contractions were obtained with the serum alone, so soon as the oxygen tension rose to about two atmospheres.

It follows that *the mammalian heart fed through the vessels of Thebesius and the coronary veins with blood-serum alone will maintain rhythmical contractions for hours when surrounded by oxygen at high tension.*

The ease with which this remarkable result was obtained encouraged the hope that even isolated pieces of the ventricle would beat if

fed with serum through a branch of the coronary artery. It was *a priori* almost certain that this would be the case, were the piece of ventricle supplied with serum at the normal blood-pressure. But to force serum through a coronary artery at the normal pressure requires a pressure-apparatus difficult of control in an extrinsic pressure of two atmospheres. Even were this difficulty overcome, the rate of flow through a piece of ventricle fed at fairly high pressure is rapid and a large volume of serum would be required. Now, a sufficiently large volume of serum cannot be obtained from a single animal, and it is somewhat disadvantageous to use the blood of other animals, even of the same species. It seemed best, then, to attempt perfusion at a very low arterial pressure, trusting that even this slight driving force would carry serum enough through the capillaries to produce and maintain contractions. The complete success of this undertaking is shown in the following experiment.

June 17, 1898. A cat, anæsthetized with ether, was bled from the left carotid artery, the blood defibrinated, diluted one-half with 0.8 per cent NaCl solution, and the serum separated in a centrifugal machine. An hour after the heart had ceased to beat it was removed from the chest, a cannula tied into the ramus descendens of the left coronary artery, and the part of the ventricle supplied by this branch cut away. The cannula was joined to a vessel containing 50 c.c. of the cat's serum, and placed in a glass cylinder connected with an oxygen reservoir. The height of the column of serum above the piece of ventricle was about 25 centimetres. The flow was approximately at the rate of one drop per second. The temperature was that of the room, about 25°C. The oxygen pressure was now raised to nearly two atmospheres. In a very few minutes the piece of ventricle began to beat with regularity and force, and these strong and rhythmical contractions continued so long as the supply of serum was kept up. When the serum ceased to pass, the ventricle ceased to beat.[1]

This experiment permits the further conclusion that *even isolated portions of the mammalian ventricle supplied through their nutrient arteries with a small quantity of serum at very low pressure will maintain rhythmical, long-continued, forceful contractions when surrounded by oxygen at high tension.*

[1] Similar results have been since attained with the isolated apex of the dog's heart.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

TENTH ANNUAL MEETING.

CORNELL UNIVERSITY, DECEMBER 28 and 29, 1897.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

VARIATIONS IN THE AMYLOLYTIC POWER OF SALIVA AND THEIR RELATION TO THE CHEMICAL COMPOSITION OF THE SECRETION.

By R. H. CHITTENDEN.

In some experiments conducted in 1882[1] an attempt was made to ascertain whether there is any definite relationship between the amylolytic power of human saliva and its degree of alkalinity. In the experiments then recorded, alkalinity was determined by titration with a standard acid, using cochineal as an indicator, while amylolytic power was estimated by determining the quantity of sugar formed from a definite amount of starch under given conditions. The results led to the conclusion that such variations in amylolytic power as saliva ordinarily shows, are not associated with corresponding variations in the degree of alkalinity.

In a recent paper by Hofbauer,[2] the above results are referred to with the statement that they constitute the only data recorded bearing on the amylolytic power of human saliva at different periods of the day. This statement, however, is quite misleading, for in our paper it is distinctly stated that "the saliva was collected generally an hour or two after breakfast," no attempt having been made to ascertain variations in amylolytic power for different periods of the day; indeed, in practically all of our experiments at that time, the saliva was collected at a convenient period after breakfast. The average alkalinity expressed in terms of sodium carbonate of fifty-one samples of saliva was found by the above method to be 0.08 per cent, the extremes being 0.052–0.163. We would now call attention to the fact that human saliva, while ordinarily alkaline to litmus

[1] CHITTENDEN and ELY: On the alkalinity and diastatic power of human saliva. American chemical journal, 1883, iv, p. 329.

[2] HOFBAUER, L.: Tägliche Schwankungen der Eigenschaften des Speichels. Archiv f. die ges. Physiol., 1897, lxv, p. 503.

or lacmoid, is almost invariably acid to phenolphthalëin, hence such alkalinity as it possesses, is due not to sodium carbonate but mainly to alkaline phosphates, acid phosphates being likewise present. Experiments made in our laboratory by Mr. A. N. Richards show that human mixed saliva, using lacmoid as an indicator, requires on an average 0.7 milligram H_2SO_4 to neutralize the alkalinity of 1 gram of the secretion. Expressed in terms of sodium carbonate, this would be equal to an alkalinity of 0.14 per cent. With phenolphthalëin as an indicator, on the other hand, 1 gram of saliva requires on an average 0.06 milligram NaOH to neutralize the acid salts present. It has also been found that the alkalinity as indicated by lacmoid and the acidity as indicated by phenolphthalëin are both noticeably greater in the saliva collected before breakfast than in the secretion collected after breakfast. Further, in conformity with Hofbauer's results, we find, as a rule, that the amylolytic power of saliva coming from glands which have been in a state of rest for some time, *i. e.*, collected before breakfast, is greater than that secreted an hour after breakfast. We are not inclined, however, to consider that the increased amylolytic power of saliva secreted before breakfast, for example, is to be attributed directly to the increased alkalinity, for occasional results show that amylolysis may be more pronounced with saliva having a comparatively low degree of alkalinity. The true explanation is to be found in the greater concentration of the secretion coming from the glands which have been in a state of inactivity; *i. e.*, such secretion contains a larger amount of solid matter with a corresponding increase in the proportion of amylolytic enzyme, etc. The results of a single experiment may be cited: —

Date.	Time.[1]	Alkalinity.[2]	Amylolytic power.[3]	Solids.	Organic matter.	Salts.
Nov. 24.	7.10–7.30 A.M.	0.163%	651.0	0.86%	0.58%	0.28%
"	9.00–9.30 "	0.112	615.6	0.51	0.30	0.21

Numerous results similar to the above testify to the truth of the foregoing statement. Somewhat noticeable also is the influence of

[1] Before and after breakfast.

[2] Determined by $\frac{1}{10}$ normal H_2SO_4 with lacmoid as an indicator and expressed as sodium carbonate.

[3] Expressed as milligrams of maltose formed from 1 gram of starch.

different stimuli upon the amylolytic power and chemical composition of human saliva. Experiments have been made with ether and chloroform vapor, alcohol, whiskey, and gin, the secretion obtained under their influence being compared with that resulting from mechanical stimulation, etc. The results thus far obtained tend to show that the above agents cause the secretion of a fluid richer in amylolytic enzyme and having a higher content of solid matter. The details of the experiments will be published in the next number of this Journal.

ON METABOLISM IN FATTY DEGENERATION.

By GRAHAM LUSK.

MANY years ago Voit declared his belief in a preliminary cleavage of the proteid molecule within the organism into a nitrogenous portion and a non-nitrogenous portion, which were subsequently burned within the cells, often at different times. To the non-nitrogenous portion belonged the sugar of the starving diabetic, and it likewise furnished fat in fatty degeneration. Through the subcutaneous injection of phlorhizin in dogs a ratio of sugar to nitrogen as 3.75 is to 1 has been established in the laboratory of the writer. This signifies that the proteid molecule may yield 60 per cent of dextrose. Accompanying this intense form of diabetes may be seen in the starving dog a rise of 450 per cent in the proteid decomposition, an effect probably due to the non-combustion of the sugar produced. The only case parallel to this in the extent of its proteid decomposition lies in phosphorus poisoning, where a similar increase is present. The question arises, is not this high proteid metabolism in phosphorus poisoning likewise due to the non-burning of the sugars, consequent upon their quantitative conversion into fat? In other words, may not the 60 grams of dextrose obtainable from every 100 grams of proteid be converted into fat in cases of acute fatty degeneration?

In a first experiment upon a diabetic dog the ratio in the urine was found to be Dextrose : Nitrogen = 3.75 : 1. During the administration of the phlorhizin, phosphorus oil was also given, with the idea of reducing possibly the sugar in the urine by means of its conversion into fat. No decrease in the sugar followed, although the dog died with every symptom of phosphorus poisoning. This experiment,

however, does not disprove the idea that in fatty degeneration the sugar from proteid is converted into fat, for the phlorhizin may have protected the sugar immediately upon its formation from any further change. A second experiment made the subject clearer. A starving dog was poisoned with phosphorus; all the symptoms, including a high rise in proteid metabolism, were manifest. Under these circumstances, if proteid sugar is being converted into fat there should be no sugar present in the body. Now, the action of phlorhizin is first to sweep the body clear of sugar, as is indicated by the high ratio of sugar to nitrogen observed always on the first day of phlorhizin administration, even after long fasting. If now in the dog poisoned with phosphorus no sugar was present and we administered phlorhizin, no excess of sugar should be eliminated; only that belonging to the proteid decomposition for the time being should be eliminated. The result obtained conformed with this theoretical expectation. The ratio in the urine was Dextrose: Nitrogen = 3.65 : 1. This indicates that in phosphorus poisoning there is no sugar present in the dog. Either one of two conditions may here be possible: either the sugar is burned as soon as formed, or it is converted into another substance. That it is immediately burned is improbable on account of the high proteid metabolism; — its burning would reduce proteid metabolism. The sugar must, therefore, have been converted into another substance or into fat. It seems reasonable to conclude that in acute fatty metamorphosis of the cell the dextrose formed from proteid in the cytoplasm may be quantitatively converted into fat.

THE ACTION OF THE LARYNX IN THE PRODUCTION OF VOICE.

By W. HALLOCK (with F. S. MUCKEY).

THE organ of voice-production is essentially a string, not a reed instrument. The two fundamental reasons for this conclusion are: first, the agencies for the control of pitch are the agencies that control the pitch of a string, namely, tension, length, and weight; secondly, the quality of the tone produced is the quality of the tone of a string.

Voice-production and voice-modification (articulation) are managed by distinct, independent sets of muscles, the former by the intrinsic

laryngeal muscles, the latter by the extrinsic muscles; and neither set should be permitted to usurp or interfere with the functions of the other.

In the correct production of voice there should be no registers. The three agencies for the control of pitch are mediated by the intrinsic laryngeal muscles only. They should act simultaneously, independently, evenly, and gradually, and produce a smooth and continuous rise in pitch from the lowest tone to the highest, the action and operation of the larynx being the same throughout. If the extrinsic muscles are allowed to come into action and pull upon the larynx, the latter is distorted and the delicate action of the arytenoid cartilages is absolutely blocked. It then becomes necessary to rely entirely on change of tension to control pitch, and of the three factors this is the most difficult of control, because the pitch is directly proportional to only the square root of the tension of the cords, whereas it is inversely proportional to the length and weight. Under these conditions registers arise, owing to the imperfect coöperation of and coördination between the intrinsic and extrinsic muscles, and the cords are seriously strained by the high tension to which they must be submitted in the effort to produce the high tones. This abnormal strain results in impairment of the muscle-structure, and then in faulty approximation of the vocal bands, with all the evil consequences thereof. The most pernicious of all habits in voice-production is this of permitting the large and powerful extrinsic muscles to usurp the duties of the delicate intrinsic muscles and prevent their action, while unable themselves to accomplish the same results.

The classic investigations of Helmholtz, König, and many others, have proved that in the human voice the sound consists of a fundamental or pitch tone, accompanied by one or more of a series of overtones, the quality of the voice being dependent upon the latter. By photographing the movements of sensitive flames we have been able to analyze tones and thus to verify completely the general correctness of the visual and oral observations of Helmholtz and Konig. Our photographs give an impersonal impartial record of the "string overtones" in the voice, and their modification of quality, not only in different voices, but in different vowel sounds in the same voice.

In order to reinforce a tone, a cavity must have a fixed size, shape, and opening. The vibrations must be able to pass in as well as out at the opening. Reinforcement by chest-resonance is impossible, for two reasons especially: the chest is a cavity of varying size even

during a single breath, and it is essentially a closed cavity. The air in the chest may, and does vibrate, — so it does in the wind-box of an organ, — but these vibrations cannot reinforce the tone produced externally. The antra and sinuses are also useless for resonant reinforcement.

DEMONSTRATION: ETHER-ANÆSTHESIA BY THE RECTUM.

By S. J. MELTZER.

A SMALL bottle half filled with ether, and closed with a cork perforated by a glass tube, was placed in a water-bath at a temperature above the boiling point of ether. The ether vapor generated was led into the rectum by means of a metal tube provided with a number of side openings, besides an aperture at the end, and connected with the ether bottle by means of rubber tubing. As ether boils at a point below the temperature of the body (about 35° C.), the introduced vapor remains in the intestinal canal in a gaseous state, and is there readily absorbed. The rate of absorption in this place is by no means comparable with that of the absorption of ether by the lungs. The absorption, however, is greatly facilitated by an increase of the intra-intestinal pressure, which can be easily accomplished by increasing the temperature of the water-bath, and thus introducing rapidly large quantities of ether vapor into the intestines. It must be borne in mind that the favorable as well as the dangerous state of anæsthesia depends upon the amount of ether present at one time in the blood, and this depends not only upon the rate of absorption, but also upon the rate of the excretion from the body. Ether is always excreted through the lungs. If too large quantities of ether vapor are rapidly thrown into the intestines, not only will absorption be increased, but the enormously developing meteorism may seriously impair the respiration, and considerably diminish the excretion of the ether, and thus cause death. On the other hand, the excretion of ether by the lungs is apparently more completely accomplished when the vapor is introduced into the rectum than when inhaled by the lungs, as in the latter case the ether has to be exhaled into an atmosphere already saturated with ether. If the temperature of the water is not too high, and if care is taken to remove frequently the ether bottle from the water-bath, the ether anæsthesia by the rectum is a safe and convenient method for certain laboratory purposes. Thus, a rab-

bit can be narcotized in a few minutes, and can be kept in a state of anæsthesia for many hours without the aid of a special assistant. The peristalsis usually removes the surplus of gas from the intestines, if the gas is not generated too rapidly, and a moderate meteorism can easily be removed by gentle massage. The absorption seems to take place in the rectum, at least there was no ether present in the small intestines in cases of complete anæsthesia from a moderate generation of ether vapor. In dogs between twenty minutes and half an hour is required for a thorough anæsthesia. But during this period there seems to be no danger whatsoever for the animal. An injection of morphine facilitates the result without increasing the danger. The rectal tube should be fastened so as to prevent its expulsion by the peristalsis, and the ether bottle should be kept higher than the rectum, in order to prevent the contamination of the ether by intestinal contents.

Anæsthesia by the rectum has the following advantages: If performed properly it is by far less dangerous to the animal than anæsthesia by inhalation. It requires little attention, and no special assistant. It does away with all the reflexes affecting respiration, heart-beat, and blood-pressure, which are such disturbing elements in anæsthesia by inhalation.

Rectal anæsthesia was suggested by Pirogoff for surgical operations as early as 1849. It did not come into practical use until the beginning of the eighties, when it was tried abroad and in this country. The slow procedure, the tenesmus, and the possibility of meteorism prevented its general use. Some experiments were made on animals, but only for testing its practicability for surgical purposes. So far as I know, no previous attempt to introduce it for laboratory purposes, has been made.

DEMONSTRATION: A SIMPLE METHOD FOR THE REDISTENTION OF THE COLLAPSED LUNG.

By S. J. MELTZER.

THE method mostly employed for the redistention of the collapsed lung (in animals) is the sucking out of the air from the pleural cavity. But any one who has had extensive experience with this method knows how unsatisfactory it is. In many cases a piece of the lung is firmly sucked into the cannula, or if the opening in the chest is

made in the sixth intercostal space, or lower, it often happens that even the diaphragm is sucked into it. With the aid of my pleural cannula I have demonstrated on a rabbit a simple method for the redistention of the collapsed lung, and the re-establishing of negative pressure in the pleural cavity. The protruding nozzle of the cannula is connected with a Müller's valve. Then the hand is placed upon the abdomen, and the stomach and the liver are pressed into the thorax while the trachea is being compressed. As the air of the compressed, non-collapsed lung cannot escape through the trachea, it enters into the collapsed lung and distends it. By this distention, and by the pressure from below, the air is driven out of the perforated pleural cavity, while the valve prevents the entrance of air.

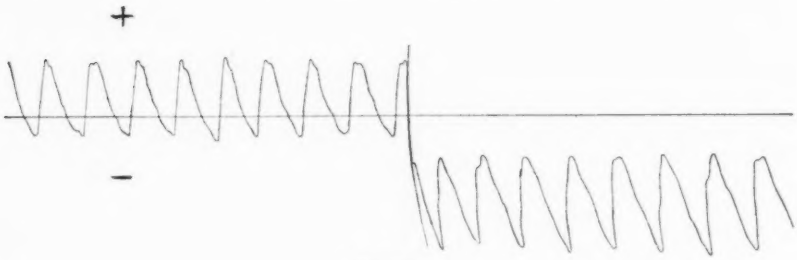

FIGURE 1.

When now the stopcock of the cannula is closed, the tube leading to the valve removed, and the nozzle connected with a manometer, the latter immediately shows a negative pressure. In the experiment illustrated by Fig. 1 the nozzle of the cannula was connected with a Marey's tambour. The straight line was drawn under normal atmospheric pressure; all above the line is at positive, and all below at negative pressure. The undulations at the left were obtained from the pleural cavity while it contained air. The expiration was always positive. Then the lung was distended, and the air driven out by the method described above, and the cannula again connected with a Marey's tambour. Both expiration and inspiration were now below the line of the atmospheric pressure.

ON CERTAIN CHARACTERISTICS OF THE PRESSURE SENSATIONS OF THE HUMAN SKIN.

By G. P. CLARK.

VON FREY has shown that the effectiveness of non-painful mechanical stimuli, in exciting the so-called sense of pressure of the human skin, depends upon certain factors in addition to the strength of the stimulus, namely, the rapidity of its application, the size of the surface to which it is applied, and the locality of the skin stimulated. He determines the value of the physiological factor, the so-called "pressure-points," of any skin surface, by the use of test-hairs (*Reizhaare*), the pressure of which is calculated from careful measurements of the applied surface and the power, *i. e.*, the weight which each can balance on the scales. Finding that test-hairs of greater surface and power are more effective physiologically than those of smaller surface and power, but of the same hydrostatic pressure, he assumes that the nerve organs concerned in the pressure sense are situated somewhat deeply in the skin. An object of the research here reported was to determine whether the same organs in the skin, which have been shown to be called into action by the deformation caused by pressure (*Druck*), are also excited by that caused by traction (*Zug*), or whether other organs are concerned. The movements of the structures underneath the skin may evidently change the tissue pressure of the skin, either increasing or diminishing it, according to the kind of movement and the relation of the skin to the part moved. Changes of pressure, corresponding to those of pressure or traction from without upon the surface of the skin, may thus arise. Tests were made upon very small (0.3 to 0.5 mm^2.) and large (10 to 50 mm^2.) surfaces on the left wrist and thumb, and with momentary and continued stimuli of different strengths. The stimuli were applied by means of a double-arm wooden lever in equilibrium, the end of one arm being connected by a very light straw with the surface of the skin to be stimulated, the end of the straw, or of a cork disc, which was slipped on to it when increase of surface was desired, being glued to the skin. The forearm of the person upon whom the tests were made was held in a plaster of Paris mould. Weighting or striking the arm of the lever between its axis and the skin served to produce pressure; weighting or striking the opposite arm produced traction. It was found that the so-called

"pressure-points" most sensitive to pressure are also most and equally sensitive to traction; that with very small surfaces (0.3 mm^2.) there is inability to distinguish between pressure and traction, even with strong and continued stimuli; and that fatigue produced by a strong continued pressure stimulus is fatigue for effects of subsequent momentary traction as well as pressure stimuli. With large surfaces (50 mm^2.) it was found that with momentary stimuli, even of marked strength, there is inability to distinguish between pressure and traction, and that continued stimuli may be of insufficient strength to enable one to distinguish those of pressure from those of traction.

Collectively the tests showed that ability to distinguish between pressure and traction depends upon the size of the surface stimulated, the duration of the stimulus, and the strength of the stimulus; that it is not an inherent quality of the impulse excited in the nerve organs of the skin by the changes of pressure. It having been found that the points most sensitive to pressure are also most sensitive to traction; that simple sensations of deformation are provoked by simple stimuli in either direction; that fatigue for pressure is also fatigue for traction; and that the factors, strength of stimulus, rapidity of application, size of surface to which the stimulus is applied, and locality of skin stimulated are of the same value in the effectiveness of traction stimuli as they have previously been found to be in that of pressure stimuli; — it is assumed that the same nerve organs in the skin are excited by both kinds of stimuli.

THE MOVEMENT OF FOOD IN DEGLUTITION.

By A. MOSER and W. B. CANNON.
[Reported for H. P. BOWDITCH by W. T. PORTER.]

By mixing subnitrate of bismuth with the bolus, the passage of the food along the œsophagus can be seen with the Roentgen rays. In the cat, solid and mushy boluses are carried down by peristalsis, the descent being more rapid in the upper thoracic region than in the neck or below the level of the heart. Liquids descend faster than solids or soft solids as far as the level of the heart, but often remain there for several minutes before a peristaltic wave pushes them into the stomach. In man, solids and soft solids are likewise forced down the œsophagus by peristalsis.

THE MOVEMENTS OF THE STOMACH, STUDIED BY MEANS OF THE ROENTGEN RAYS.

By W. B. CANNON.

[Reported for H. P. BOWDITCH by W. T. PORTER.]

THE conclusions reached in this investigation are as follows: (1) By mixing a harmless powder, subnitrate of bismuth, with the food, the movements of the stomach can be seen by means of the Roentgen rays.

(2) The stomach consists of two physiologically distinct parts: the pyloric part and the fundus: over the pyloric part, while food is present, constriction-waves are seen continually coursing towards the pylorus; the fundus is an active reservoir for the food, and squeezes out its contents gradually into the pyloric part.

(3) The stomach is emptied by the formation, between the fundus and the antrum, of a tube along which constrictions pass. The contents of the fundus are pressed into the tube, and the tube and antrum are slowly cleared of food by the waves of constriction.

(4) The food in the fundus is not moved by peristalsis, and consequently it is not mixed with the gastric juice; it can therefore undergo salivary digestion in this region for a considerable period without being disturbed. The food in the pyloric portion is first pushed forward by the running wave, and then by pressure of the stomach wall is returned through the ring of constriction; thus the food is thoroughly mixed with gastric juice and is forced by an oscillating progress to the pylorus.

(5) The pylorus does not open at the approach of every wave, but only at irregular intervals. The arrival of a hard morsel causes the sphincter to close tightly, thus materially interfering with the passage of the already liquified food.

(6) Solid food remains in the antrum to be rubbed by the constrictions until triturated, or to be softened by the gastric juice, or later it may be forced into the intestine in the solid state.

(7) The constriction-waves have, therefore, three functions: the mixing, trituration, and expulsion of the food.

(8) At the beginning of the act of vomiting the gastric cavity is separated into two parts by a constriction at the beginning of the antrum; the cardiac portion is relaxed and the spasmodic contractions of the abdominal muscles force the food through the opened cardia into the œsophagus.

(9) The stomach movements are inhibited whenever the animal shows signs of anxiety, rage, or distress.

The full paper will be published in the next number of this Journal.

NEW EXPERIMENTS ON THE MAMMALIAN HEART.

By W. T. PORTER.

I. The recovery of the whole heart from fibrillary contractions; see this Journal, vol. i, page 71.

II. The effect of the beat of the heart upon the flow of blood through the walls of the heart; see this Journal, vol. i, page 145.

III. A method for the study of the blood-currents at the root of the aorta.

A small cylinder, covered with lead foil, and of the same specific gravity as the blood, is fastened by a very short thread to the end of a probe and passed through the carotid artery and aorta to a position just above the semilunar valves. The movements of the cylinder are those of an equal mass of blood. They may be watched with the Roentgen rays after the removal of the ribs.

THE EFFECT OF INANITION ON THE STRUCTURE OF NERVE CELLS.

By F. W. BARROWS.

THE researches to be described were undertaken in order to find out by what structural alterations, if any, a starved nerve cell may be distinguished from one that is well nourished.

In each of three experiments, three rats of the same sex, and similar in weight and general condition, were kept in mechanical cages side by side. Kymograph records gave a continuous history of the activities of each animal during the experiments, together with the temperature and atmospheric pressure for each moment of time. A study of these records shows that fatigue as well as starvation is a strong factor in producing the effects noted. Upon the death of the famished rat, the control rat was weighed and killed. The tissues of the famished and control animals selected for comparison were treated together in the manner described by Dr. Hodge in his work on Fatigue. By this method, the tissues of the normal and famished

animals received exactly the same treatment from the moment of dissection until they were mounted together on the same slide. Microscopical comparison and measurement of normal and famished nerve cells from the occipital cortex, spinal ganglia, and cord, shows: —

(1) A decided shrinkage in size of the cells and nuclei in the famished animals, averaging about 20 per cent, and a still greater shrinkage in the nucleoli.

(2) An evident exhaustion of the substance of famished cells, as shown by their faint staining with osmic acid and the notable absence of nuclei and nucleoli. The protoplasm of these cells shows a very fine vacuolation, not so marked as that described by Rosenbach for starving animals, and by Hodge for extreme fatigue. In the brains of famished rats the pericellular lymph spaces are considerably enlarged.

The Composition and Nutritive Value of Some Edible American Fungi. By Lafayette B. Mendel.

See this Journal. vol. i, p. 225.

Some Experiments on the Excretion of Kynurenic Acid. By L. B. Mendel.

Demonstration: A New Pleural Cannula *in situ*. By S. J. Meltzer.

A description of the cannula will be published in this Journal.

Demonstration: The Nutrition of the Mammalian Heart through the Vessels of Thebesius. By W. T. Porter (for F. H. Pratt).

See this Journal, vol. i, p. 86.

A Basis for a Theory of Color Vision. By W. Patten.

Influence of Alcohol upon the Young in Dogs and upon the Severity of an Attack of Distemper. By C. F. Hodge.

Read by title.

Influence of Alcohol upon Voluntary Muscular Power in Conditions of Fatigue. By C. F. Hodge.

Read by title.

The Influence of Bile and Bile Salts on Pancreatic Proteolysis. By R. H. Chittenden.

Read by title. This paper will appear in the next issue of this Journal.

The Biological Problems of To-Day: Physiology. By J. Loeb.